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PROTOCOL DEVELOPMENT FOR THE PREDICTION OF THE FATE OF ORGANIC PRIORITY POLLUTANTS IN BIOLOGICAL WASTEWATER TREATMENT SYSTEMS

by

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16. ABSTRACT

Research was conducted on biodegradability of toxic and/or hazardous organic priority pollutant compounds. The studies have produced a biodegradation testing protocol to provide a scientific basis for predicting the fate of these compounds in typical activated sludge treatment plants that employ anaerobic digestion as a method of solids stabilization. The biodegradation protocol includes a three-level approach for aerobic studies: Level 1--acclimation and abiotic removal; Level 2--biodegradation kinetics; and Level 3--extent of biodegradation. A two-level anaerobic approach, consisting of Level 1 and Level 2 and operational performance, was also tested.

The testing procedure focuses on the study of (1) biodegradability of substrate including kinetics and effluent quality as a function of solids retention time; (2) partitioning and intermedia transport including volatilization and sorption; and (3) toxicity to activated sludge systems and to anaerobic digestion.

The report describes the evolution of the biodegradation testing protocol for the purpose of establishing quick response capability, the development of necessary laboratory procedures, the results of the application of these tests to pentachlorophenol (PCP), and the use of these data to evaluate and predict the fate of PCP in biological waste treatment systems.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxics Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Laboratory is that component of EPA's research and development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; with establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and with assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product use. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

The 20th century technical revolution has resulted in the manufacture and use of a large variety of xenobiotic chemicals that find their way into wastewater. Since some of these chemicals have biotoxic properties at low concentrations, a select group, the priority pollutants, has been singled out for intensive review. This publication describes a carefully considered experimental approach to predicting the fate of selected priority pollutants in aerobic and anaerobic biodestruct systems--specifically, a rigorous evaluation of the experimental protocol using sodium pentachlorophenate as the model priority pollutant. In so doing, this report is intended to provide guidance to those who are concerned with the effectiveness of conventional wastewater treatment methodology to deal with the problems of hazardous wastewater management.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

Many of the organic chemicals classifed as priority pollutants can be present in the wastewater entering municipal and industrial biological wastewater treatment plants. A testing protocol has been developed to provide a scientific basis for predicting the fate of these pollutants in typical activated sludge treatment plants which employ anaerobic digestion as a method of solids stabilization.

The testing procedure focuses on the study of: I. Toxicity to activated sludge systems and anaerobic digestion; II. Partitioning and intermedia transport including volatilization and sorption; III. Biodegradability including kinetics and effluent quality as a function of solids retention time.

This report describes the evolution of the testing protocol, the development of necessary laboratory procedures and the results of the application of these data to evaluate and predict the fate of PCP in biological wastewater treatment.

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LIST OF ABBREVIATIONS AND SYMBOLS

COD --Chemical oxygen demand.
CPM --Counts per minute.
CSTR --Continuously stirred tank reactor.
d --Decay rate constant for the mixed microbial culture.
d_s --Decay rate constant for organisms carrying out degradation of the specific pollutant.
F --The liquid flow rate (L^3/T).
GC --Gas chromatograph.
HRT --Hydraulic retention time.
K_La --Volumetric mass transfer coefficient for oxygen in water.
K_s --Monod kinetic constant.
K_v --The overall volumetric mass transfer coefficient for volatilization (which incorporates the interfacial transfer area) (T^{-1}).
$K_{v\ VR}$ --K_v in the volatilization reactor.
MBAS --Methylene blue active substance.
PCP --Pentachlorophenol.
PP --Priority pollutant.
q --Mass of pollutant sorbed per mass of solids.
r_a --The rate of removal by adsorption ($M/L^3 \cdot T$).
r_b --The rate of removal by biodegradation ($M/L^3 \cdot T$).
r_v --The rate of removal by volatilization ($M/L^3 \cdot T$).
S --The pollution concentration in the reactor (M/L^3).
SCOD --Soluble chemical oxygen demand.
SCSTR --Semicontinuous stirred tank reactor.
S_e --The concentration of the priority pollutant that would be in the liquid if the liquid were in equilibrium with the air (M/L^3).
S_o --The pollutant concentration in the influent (M/L^3).
SRT --Solids retention time.
TA --Total alkalinity.
TCOD --Total chemical oxygen demand.
TS --Total solids.
TSS --Total suspended solids.
TVA --Total volatile acids.
TVS --Total volatile solids.
V --The reactor volume (L^3).
VSS --Volatile suspended solids.
X_s --The concentration of organisms responsible for degradation of the pollutant.
Y_g --The true growth yield (M/M).
Y_s --The true yield of organisms growing on the priority pollutant.
τ --The reactor liquid residence time (V/F).
μ --Specific growth rate.
μ_m --Maximum growth rate: Monod.

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CHAPTER 1

INTRODUCTION

The classification as priority pollutants of approximately 129 extensively manufactured and widely used chemicals, together with the observation that many of these chemicals can be detected in wastewater, leads naturally to the question of their fate in biological wastewater treatment systems. Since hazard evaluation of a chemical is related to its concentration and time of exposure, the extent of biodestruction in such treatment systems will probably be one of the factors affecting regulatory decisions on the manufacture and use of organic substances which manifest some toxic or hazardous potential for man and the environment. Unfortunately, the fate of many such priority pollutants in biological wastewater treatment systems has not been adequately investigated. Consequently, a research program was initiated with the goal of obtaining experimental data which would allow prediction of the fate of organic priority pollutants in aerobic and anaerobic biological wastewater treatment systems.

Because of the large number of organic chemicals on the priority pollutant list and because the number will continue to grow by new additions from the inventory of existing industrial chemicals as well as by additions from newly developed chemicals, an experimental program to develop data relevant to their fate in treatment systems is a large undertaking requiring a major commitment of time and resources. Furthermore, the economic and ecological consequences of decisions made from that data can have a significant impact upon our nation. Consequently, the experimental protocol for such a program must be conceived in a manner that will allow a thorough and accurate evaluation of the fate of the chemical under study. In short, before such a long-term research commitment can be undertaken, three questions must be addressed: 1) What sort of information must be obtained to evaluate the fate of a given chemical in the highly complex milieux of a wastewater treatment system? 2) How should this information be obtained? 3) How can this information be used to predict the fate of a particular compound? This report: 1) examines those questions within an experimental framework whose bounds were established by the operational limits of existing secondary watewater treatment facilities; 2) describes the development of a systematic experimental approach for evaluating the fate of organic chemicals in such facilities; 3) relates experiences with the test methodology selected; and 4) evaluates the application of the experimental protocol to the testing of a "typical" priority pollutant.

Pentachlorophenol (PCP) was chosen as the "typical" priority pollutant because it is known to exhibit relatively high resistance to biodegradation

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in unacclimated systems but is substantially degraded in acclimated systems. Furthermore, it shows significant bio-inhibitory properties even in systems which lead to its eventual decomposition. These considerations implied that the use of PCP would be a severe test for the proposed methodology.

In addition to the development and evaluation of the protocol, another objective of this project was the testing of as many priority pollutants as possible. Consequently studies were initiated on three other compounds (dimethylphthalate, bis-ethylhexylphthalate and monochlorobenzene) and at the time of preparation of this report testing of them was approximately 90% complete. Since the major objective of this research was the development and evaluation of the protocol it was decided that the clearest and most orderly presentation would result by limiting this report to the testing of PCP. Consequently Volume 2 will cover the evaluation of the fate of dimethylphthalate, bis-ethylhexylphthalate and monochlorobenzene.

CHAPTER 2

CONCLUSIONS

1. An experimental protocol for evaluating the fate of priority pollutants in aerobic and anaerobic wastewater treatment systems was developed.

2. The protocol was tested and refined by applying it to a study of the fate of pentachlorophenol (PCP) in aerobic and anaerobic bioreactors.

AEROBIC STUDIES

3. Pentachlorophenol (PCP) was shown to be adequately biodegraded in the growth environment of aerobic Phase I testing to require Phase II testing.

4. Aerobic Phase II testing indicated quite certainly that:

   a) Substantial biodestruction of PCP occurred at nominal solids retention times (SRT's) of 3, 7, 11 and 15 days (true SRT's of 3.2, 7.8, 12.8 and 18.3 days) since input PCP concentration of 20 mg/l dropped to mean steady state concentrations of 214, 105, 68 and 65 μg/l respectively.

   b) Stable steady state operation improved with the longer SRT's. Clear evidence of substrate inhibition was exhibited by PCP at the shorter SRT's.

   c) The threshold inhibitory concentration of PCP was determined to be 350 μg/l and it is suggested that reactor instability at low SRT's is associated with transitory excursions into inhibitory PCP concentrations.

   d) The first order PCP degradation rate constant ($^{\mu}$max/ks) was shown to be 0.0017 l/μg-day.

   e) It was estimated that the lower limit for PCP concentration in a single completely mixed reactor with infinitely long SRT would be 27 μg/l at an influent concentration of 20 μg/l of PCP.

5. Neither sorption of PCP to biomass nor gas stripping were significant factors in the removal of PCP. The former contributed to less than 0.1% of the PCP loss and the latter much less than 0.17.

6. Neither soluble organic carbon nor methylene blue active substance

could be correlated with PCP biodegradation. Therefore, the use of an internal standard was discontinued.

7. Aerobic Phase III testing established that PCP undergoes significant mineralization in the complex reactor environment.

8. A comparison of the radiosotopic method and the microbial growth method indicated that either method used alone would have established that PCP is mineralized.

9. In general, the aerobic testing protocol was shown to be an effective approach in evaluating the fate and kinetics of PCP removal in aerobic wastewater treatment.

ANAEROBIC STUDIES

10. Phase I of the anaerobic protocol was found to require modification; a toxicity test was inserted to determine inhibitory levels of the priority pollutants prior to acclimation.

11. The toxicity test suggested that a soluble concentration of PCP in excess of 200 µg/l initiates significant inhibition of gas production by unacclimated organisms. Consequently, the acclimation feeding schedule was adjusted to minimize inhibition.

12. Phase I anaerobic reactors were successfully acclimated with no apparent toxicity up to an input level of PCP that eventually reached 7.6 mg/l.

13. Significant biological removal of PCP was observed during Phase I anaerobic testing and was accompanied by the appearance of PCP-related decomposition products in the gas chromatograms.

14. Direct determination of sorption of PCP to digester sludge by solvent extraction and G.C. analysis indicated that less than 2% of the applied PCP was bound to the sludge.

15. Phase II anaerobic testing with reactors at 10, 20, and 40 days SRT's and input on PCP concentration 5 mg/l resulted in normal digester performance when compared with unspiked controls.

16. During steady state Phase II anaerobic testing soluble PCP concentrations at all SRT's tested were almost undetectable (< 10 µg/l), however, oscillations in PCP concentration and gas production were observed earlier in the testing period.

17. It appears the PCP feed concentration of less than 5 mg/l would not significantly effect anaerobic digestion if digesters are operated within the SRT limits tested. However, it is probable that shock loads of PCP would result in essentially irreversible inhibition of digestion.

18. Overall, the anaerobic testing protocol presented was shown to be an effective approach in evaluating both the fate of PCP in anaerobic digesters and the influence of PCP on "normal" anaerobic digestion.

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CHAPTER 3

RECOMMENDATIONS

1. Evaluate the protocol on other priority pollutants with different physical properties such as solubility, vapor pressure, octanol partition coefficient; and different biodegradability and inhibitory characteristics. At the time of writing, this is being done with dimethylphthalate, bis-ethylhexylthalate and monochlorobenzene. Studies with toluene and naphthalene are planned.

2. Develop full scale confirmation of the predictive capability of the protocol at several municipal and industrial wastewater treatment plants which currently receive certain of the priority pollutants.

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CHAPTER 4

TESTING PROTOCOL

The fate of an organic chemical in a wastewater treatment system is determined by the interrelated effects of biological and nonbiological elimination mechanism, commonly referred to as biodegradation and results in the conversion of the compound into another form, hopefully an innocuous one. The major nonbiological elimination mechanisms, namely sorption and volatilization, generally result in the removal of the chemical in unaltered form from the aqueous phase by transfer to either a solid or gaseous phase. All of these mechanisms are of potential importance but the actual importance of each depends upon the physical layout and characteristics of a particular wastewater treatment system. Consequently the prediction of the fate of an organic chemical depends upon knowledge of the intrinsic kinetics associated with each mechanism and the incorporation of those kinetics into mass balance equations which reflect the physical characteristics of the system of interest. Thus, in the most elementary sense, the information which must be obtained is that which allows evaluation of the kinetics of the various elimination mechanisms.

While it is relatively easy to identify the type of information needed to predict the fate of a specific organic chemical in a wastewater treatment system, it is difficult to get scientists or engineers to agree on the technique for obtaining it, if the discussions at two recent symposia (1, 2) are indicative of the current state of the art. Furthermore, there is an extensive historical literature associated with each of the elimination mechanisms and the experience represented by that literature must be considered. The protocol proposed herein is presented as a workable one, as the results which follow will show. Its development was based upon the rationale which follows, with due regard for the work of those who have preceded us. In the interest of brevity and for the sake of those who are familiar with past attempts to determine the fate of organic chemicals in wastewater treatment systems, it was decided to forego a lengthy presentation of the historical literature. Those who desire more information on alternative procedures are directed to the papers at the aforementaioned symposia (1, 2) as well as to the literature reviews in the theses upon which this report is based (3, 4).

RATIONALE

Required Information and Its Use

Basically, the information needed to evaluate the fate of an organic compound in a biological wastewater treatment system can be summarized in

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one question: What are the rate expressions for the elimination mechanisms? If those expressions were known it would then be possible to write the mass balance equations describing a particular system, thereby allowing prediction of the fate of the compound in that system. Conceptually, this is a relatively straight-forward matter, as will be demonstrated in this section. The problems arise in the techniques required to obtain the needed data because they generate an entire subset of required information.

The need for rate data can best be illustrated by considering a control volume of fluid into which and from which a liquid stream containing a priority pollutant is flowing. Within that control volume biodegradation will be occuring so that a portion of the priority pollutant is destroyed (or at least altered in form). Furthermore, if a gas stream is passing through the volume, some of the pollutant will be transferred from the liquid phase to the gas phase thereby exiting from the control volume with it. Finally, if solids enter (or are formed in) the control volume, some of the pollutant may leave the reactor associated with those solids. The concentration of the pollutant leaving the control volume will depend upon the relative rates of each of these mechanisms and the time that the liquid spends in the control volume. If the control volume constitutes the entire reactor volume, as it does with a constant volume, continuous stirred tank reactor (CSTR), then a mass balance on the priority pollutant would be:

$$\frac{Vds}{dt} = FS_o - FS - r_bV - r_vV - r_aV \qquad (1)$$

where S_o is the pollutant concentration in the influent (M/L^3),

S is the pollutant concentration in the reactor (M/L^3),

V is the reactor volume (L^3)

F is the liquid flow rate (L^3/T),

$-r_b$ is the rate of removal by biodegradation $(M/L^3 \cdot T)$,

$-r_v$ is the rate of removal by volatilization $(M/L^3 \cdot T)$,

$-r_a$ is the rate of removal by adsorption $(M/L^3 \cdot T)$.

If the reactor is at steady state the derivative term may be set equal to zero, thereby giving an algebraic expression for the concentration of pollutant leaving the reactor:

$$S = S_o - (r_b + r_v + r_a)\tau \qquad (2)$$

where τ is the reactor liquid residence time (V/F).

If the reactor is not a single CSTR, its hydraulic characteristics can probably be approximated as a series of n CSTR's and thus the performance of the real reactor can be estimated by solving n sequential equations, each like Eq. (2) in form. If recycle is employed around the chain, the equations must be solved iteratively.

If the reactor cannot be assumed to be at steady state, then the differential equation (Eq.1) must be solved to obtain the time-dependent performance of a single CSTR. In practice this is difficult to accomplish because the state of the art in kinetic modeling of biodegradation is not well developed for the dynamic case, particularly not the inhibitory material. The dynamic modeling of microbial growth and substrate utilization is an entire research field by itself and consequently it was decided to limit this work to the steady-state case. Furthermore, since the prediction of the performance of a short series of CSTR's is simply an extension of the single reactor case, it was decided to limit this work to single reactors. If the reaction rate expressions are for the intrinsic kinetics of the reactions, the expressions will be the same whether the reactor is a single CSTR or a CSTR to which cell recycle has been applied. Consequently, it was decided to conduct experiments to determine the rate equation, $-r_b$, in single, simple CSTR's (the practical reasons for doing this will be discussed later). Once the rate equation has been determined it can be applied to the equation for a CSTR with cell recycle to predict the fate of the pollutant in an activated sludge system. Those desiring more information on this topic should consult Grady and Lim (5).

REMOVAL BY BIODEGRADATION--

In spite of the fact that wastewater treatment engineers have been working for years with multicomponent substrate systems, relatively little is known about the rates of biodegradation of individual organic species in such systems. Bader (6) has discussed the rate expressions which might be used for such compounds and has divided them into two categories: interactive and noninteractive. For the purpose of this project it was decided to try the noninteractive approach first, because it was the most straight-forward. Attempts to use the interactive approach would only be used if the noninteractive approach failed. As will be seen later, the noninteractive approach was sufficient for this first attempt. Basically, it assumes that the specific growth rate of the microorganisms responsible for the degradation of a priority pollutant is a function of the concentration of that pollutant alone:

$$\mu = f(S) \tag{3}$$

Many functional forms could be proposed for Eq. (3), but common practice and precedence suggest that the Monod equation (see ref. 5) is a reasonable beginning point. Thus

$$\mu = \frac{\mu_m S}{K_s + S} \tag{4}$$

where μ_m and K_s are empirically determined parameters describing the hyperbolic relationship. For an inhibitory substrate a more complicated equation could be used, as suggested by Andrews (7). For steady-state growth, however, the only stable steady-states are those expressed with Eq. (4) and thus it should be adequate for now.

The advantage to using the noninteractive approach is that it implies that the concentration of the priority pollutant in a steady-state CSTR is governed solely by the specific growth rate of the organisms responsible for its degradation. Furthermore, as long as the organisms responsible for its degradation are homogeneously interspersed with the other biomass their specific growth rate is controlled by the solids retention time (SRT) of the reactor (5):

$$\mu = (1/SRT) + d_s \tag{5}$$

Where d_s is the decay rate constant for the organisms carrying out degradation of the pollutant. Combining Eqs. (4) and (5) shows that the steady-state concentration of the pollutant in a CSTR is a function of only the kinetic parameters describing its degradation and the SRT of the reactor:

$$S = \frac{K_s\ [(1/SRT) + d_s]}{\mu_m - [(1/SRT) + d_s]} \tag{6}$$

This suggests that Eq. (2) is not needed to find the steady-state concentration of the pollutant in the reactor. The reason for this can be seen in the following argument. The rate of substrate removal by biodegradation is given by

$$-r_b = \frac{\mu X_s}{Y_s} \tag{7}$$

Where Y_s is the true yield of organisms growing on the priority pollutant, and X_s is the concentration of organisms responsible for degradation of the pollutant. If Eq. (7) and Eq. (5) are substituted into Eq. (2) and the result is rearranged, we obtain

$$X_s = \frac{Y_s\ (S_o - S)}{[(1/SRT) + d_s]\tau} - \frac{Y_s\ (r_v + r_a)}{[(1/SRT) + d_s]} \tag{8}$$

Thus it can be recognized that the sole effect of the nonbiological elimination mechanisms is to reduce the concentration of organisms within the reactor which are responsible for degradation of the pollutant. Thus once μ_m, K_s and d_s are known, the concentration of pollutant in a reactor may be predicted for any SRT value.

It should be noted that the above argument applies only to a single steady-state CSTR with or without cell recycle and to a chain of steady-state CSTR's without cell recycle (which will have a slightly different equation for μ and which must be solved sequentially down the chain). It does not apply to a chain of steady-state CSTR's with cell recycle around the entire chain or to any nonsteady-state CSTR's. The reason for this is the Eq. (5) comes from a steady-state mass balance on cells in a single CSTR (with or without recycle). For the case of a chain of CSTR's with recycle around the entire chain mass balances must be written on each reactor for the pollutant and the cells responsible for its degradation and then this family of equations must be solved iteratively for the values of S and X_s in each tank. A similar approach must be used for the nonsteady-state case. In that situation knowledge is required of Y_s and the rate expressions for the nonbiological elimination mechanisms (r_v and r_a) before S can be found. The solution techniques for this situation are straight-forward and will not be discussed further.

REMOVAL BY VOLATILIZATION--

Removal by volatilization is a mass transfer event and thus the rate of removal of the pollutant will be equal to the product of a mass transfer coefficient and a driving gradient. By analogy with oxygen transfer, an appropriate mass transfer coefficient would be the overall one and the corresponding driving gradient would be the difference between the concentration of the priority pollutant in the liquid and the concentration that would be in the liquid if it were in equilibrium with the air being used for aeration:

$$r_v = K_v (S - S_e) \tag{9}$$

Where K_v is the overall volumetric mass transfer coefficient for volatilization (which incorporates the interfacial transfer area) (T^{-1}), and S_e is the concentration of the priority pollutant that would be in the liquid if the liquid were in equilibrium with the air (ML^{-3}). Maximum removal by volatilization will occur in a reactor that is being aerated by surface aeration, so that S_e is zero, or in one that is relatively shallow so that S_e is approximately zero for bubble aeration. For deep basins aerated by bubble aeration or for basins subjected to pure oxygen aeration the removal by volatilization will be less because of the effect of the partial pressure of the priority pollutant in the gaseous phase on S_e. The effects of aeration system configuration have been considered in detail by Freeman et al. (8).

Since the reactors used herein were shallow, S_e for the aerobic reactors was assumed to be zero, and Eq. (9) was simplified to read

$$r_v = K_v S \tag{10}$$

Thus, the mass transfer coefficient must be evaluated before the removal rate by volatilization can be predicted.

Once the steady-state concentration of a priority pollutant in a CSTR is know, the contribution of volatilization to its removal can be estimated simply by multiplying Eq. (10) by the reactor volume.

REMOVAL BY ADSORPTION--

Although the term adsorption is being used to represent the removal of the pollutant on (or in) the solids without degradation or metabolism, a more accurate term might be simply "sorption". This is because solids of biological origin (i.e., either micro-organisms, such as in activated sludge, or insoluble organic matter, such as in primary sewage sludge) contain lipid components into which many priority pollutants can be absorbed. Consequently when the pollutant is brought into contact with the solids, part of the removal will be due to adsorption on the surface and part will be due to adsorption into the lipid fraction. The net effect of this dual mechanism is to greatly complicate the determination of the rate expression for sorption.

The rate equation for adsorption onto a relatively homogeneous adsorbent like activated carbon is extremely complicated and difficult to obtain, expecially in a multisolute system. Furthermore, relatively little is know about the rates of absorption into the lipid component of biomass. Consequently, at the present time there does not appear to be any straight-forward approach for estimating the rate of sorption of the priority pollutant onto the solids in the reactors. Thus an alternative approach must be used.

For the cases of the steady-state single CSTR's (with or without cell recycle) and the chain of steady-state CSTR's without cell recycle the rate of adsorption is not needed in order to determine the concentration of the priority pollutant in the reactors. Consequently the primary interest in the role of sorption is to establish how much of the pollutant is leaving the reactor in the unaltered state attached to the solids. If the contact time of the liquid with the solids in the reactor is sufficiently long to allow equilibrium to be approached, then the equilibrium values can be used to do this. As will be seen later, the time required for equilibrium to be achieved when PCP is brought into contact with microbial biomass is relatively short. Thus it appears reasonable (at least for PCP) to assume that equilibrium is approached. The time required to achieve equilibrium with anaerobic sludge is considerably longer (as will be discussed later) but it is still much less than the liquid and solids residence times in anaerobic reactors. Thus it appears reasonable to assume equilibrium in that case also. Thus, the approach that can be used with the aforementioned reactors is to calculate the amount of pollutant lost by sorption by considering equilibrium to have been achieved between the concentration of PCP in solution and the mass of PCP held per unit mass of solids. This will require knowledge of the sorption isotherm in which the sorptive capacity, q, is considered to be a function of the concentration of solute in solution at equilibrium:

$$q = f(S) \qquad (11)$$

Where q = mass of pollutant sorbed per mass of solids.
The mass rate of loss of the pollutant from solution is then taken as the product of q times the mass rate of removal of solids from the reactor, which is related to the SRT.

The equilibrium approach can also be used to incorporate the contribution of sorption to the removal of pollutant from a steady-state chain of CSTR's which recycle around the entire chain by utilizing an iterative approach to the solution. For nonsteady-state situations, however, rate data are needed. Although the task of establishing a procedure for estimating the rates of sorption onto biological solids was considered to be beyond the scope of this study, it appears reasonable that an empirical approach could be used. Consequently, prediction of the fate of individual pollutants in nonsteady-state reactors must await the development of this technique. The study herein was limited to the steady-state case.

Techniques for Obtaining the Information

BIODEGRADATION--

Acclimation--The first task which must be performed in the assessment of the potential for biodegradation of a pollutant is the development of an acclimated biomass. Biodegradability is not an inherent characteristic of an organic chemical; rather it is the result of highly complex interactions among the chemical, the microorganisms brought into contact with it, and the environment in which they are placed. Consequently the success of an acclimation period is highly dependent upon the numbers and types of microorganisms brought into contact with the test compound and the growth conditions imposed upon them during that contact. It is likely that acclimation of a mixed microbial culture to a xenobiotic compound involves the following steps: the exposure to the compound of organisms which have the genetic capability to catalyze at least one reaction with the compound; exposure of other organisms to metabolic products arising from the action of the first group of organisms; expression of the genetic capabilities of those organisms through synthesis of requisite enzymes; and outgrowth in the culture of all of the organisms to levels sufficiently large to bring about degradation of the test compound at a rate sufficient to balance its rate of input. While it is recognized that mutation and the transfer of genetic information may also play a role in acclimation, it is the opinion of the authors that a realistic assessment of the biodegradability of a compound currently being released to the environment requires that those events occur spontaneously and thus no action should be taken by the investigator to increase their occurrence.

Since acclimation depends strongly upon the chance meeting of an organism bearing a particular genetic capability with an organic compound against which that capability can act, on requirement for successful acclimation is the maximization of the probability of that meeting. This can be done by starting with a high density of organisms in the acclimation reactor and by continually placing additional organisms into it. If possible, the sources of those organisms should be from places where there was a likelihood of prior exposure to the test compound. In the case of aerobic reactors, one way of achieving these goals would be to fill the reactor initially with a high density of activated sludge and to include untreated domestic sewage as a portion of the feed. For anaerobic reactors a suitable initial inoculum would include actively digesting sewage sludge mixed with bottom sediments from environments containing the test compounds. Continual inoculation can be assured by utilizing raw primary sludge as one component of the feed.

The expression of the genetic capability by the exposed organisms requires careful control over the environmental conditions. First, the test compound must be present in sufficient concentration to allow induction of the requisite enzyme(s). It appears that there is a threshold level required to bring about induction (9) although little is known about its value (in fact, the value will probably depend upon the compound). Thus, the test compound must be provided at a reasonable level. Second, the possibility of toxicity or inhibition must be considered and the chosen concentration must be low enough to avoid it. Third, because of the nature of

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the events required to achieve enzyme induction and synthesis, the culture must be actively growing. This suggests that alternative carbon and energy sources should be provided during the acclimation period. This is an important point because the fate of a compound in wastewater treatment systems is not dependent upon it serving as the sole source of carbon and energy for the organisms present. Instead, it is likely that degradation of some xenobiotics (and their reaction products) may occur by cometabolism. Thus the provision of alternative carbon and energy sources provides an opportunity for the maximum number of potential degradation mechanisms to be utilized. The fourth requirement results from the third. If alternative carbon and energy sources are to be provided, the growth conditions of the culture must be controlled so that metabolic control mechanisms do not preclude the expression of the requisite genetic capability. This means that the specific growth rate of the culture must be maintained at a low value. This is most easily accomplished by continuous culture in a CSTR. Furthermore, carbon and energy sources should be growth-limiting and thus inorganic nutrients should be provided in excess.

Finally, after it has been established that the test compound is being eliminated from the acclimation reactor (and the occurrence of degradation is implied), conditions must be altered to allow outgrowth of the responsible organisms. This can be done by slowly increasing the concentration of the test compound in the feed while maintaining the concentration of the other carbon sources at a constant value. This has the effect of temporarily increasing the concentration of the test compound in the acclimation reactor, thereby allowing the specific growth rate of the organisms responsible for its degradation to increase in proportion to the specific growth rate of the rest of the biomass. This allows them to increase their numbers within the biomass. Their total fraction within the population will approach the limit provided by the fraction of carbon and energy provided by the test compound in the feed. If the compound has inhibitory properties, extreme care must be used in setting the rate at which the concentration in the feed can be increased. As demonstrated later, this may require experimental knowledge of the inhibitory effects of various pollutant concentrations.

In summary, an acclimation protocol should include continuous culture of the biomass on a complex carbon and energy source at a low specific growth rate with continuous addition of organisms in the feed. Acclimation should then be brought about by adding the test compound to the feed in low concentration. That concentration should be maintained until evidence is obtained for disappearance of the test compound from the reactor. After that, the concentration in the feed should be carefully increased to allow outgrowth of the acclimated organisms within the mixed microbial culture.

The ideal reactor for conducting acclimation is a completely mixed continuous flow one. Furthermore, the provision for cell recycle will allow a maximum rate of input of organisms and a minimum time constant for changes in the influent pollutant concentration while providing independent control over the specific growth rate. For the aerobic studies this can be easily accomplished with a fiber-wall reactor (10). For the anaerobic studies the necessity for feeding primary sewage sludge makes true continuous culture difficult to attain on a small scale. However, if the hydraulic retention time is long, semicontinuous culture with once-a-day feeding provides a good approximation of continuous culture. Furthermore, it would allow cell recycle by centrifugation of the effluent removed each day prior to feeding. Thus, for practical reasons semicontinuous culture offers a good compromise.

Determination of kinetics--It is widely recognized that the most effective way of establishing the kinetic relationship between the specific growth rate of a microbial culture and the concentration of a growth-limiting nutrient is through continuous culture in a CSTR (5). This follows from several facts. Most important among them are that the specific growth rate may be controlled by fixing the SRT of the reactor (Eq. (5)) and that under steady-state conditions the physiological state of the culture reaches a stable condition reflecting the concentration of substrate in the reactor. Thus operation at a number of SRT's while measuring the concentration of the compound of interest provides the information necessary to evaluate the kinetic parameters in Eq. (6). It should be recognized, however, that in a mixed microbial population where shifts in the species present can alter the content of the population attained, there are composite values which incorporate not only the effects of the growth-limiting material on the specific growth rate but the mix of organisms as well.

For the aerobic studies a CSTR with cell recycle would mimic most closely the physical situation in an activated sludge wastewater treatment plant. However, the time and effort required to accurately control SRT in such a reactor is considerable. Inherent in Eq. (5), however, is the concept that the specific growth rate in a CSTR is controlled only by the SRT and is uninfluenced by the presence or absence of cell recycle. The only difference between the two systems is that the SRT in a CSTR without recycle is equal to the hydraulic retention time (HRT) whereas in a CSTR with recycle it is independent of the HRT and dependent only upon the reactor volume and the rate of cell wastage. Consequently, the same information can be obtained from either type of reactor provided that the SRT values employed are characteristic of full-scale systems. Typical SRT values for full-scale activated sludge plants treating domestic wastewater lie between 3 and 15 days and thus it was decided to employ nonrecycle CSTR's with HRT's (and thus SRT's) between those limits. The selection of the number of SRT values requires a balance between obtaining sufficient data to evaluate the kinetic parameters and making the work load so large that it cannot be accomplished. The use of four reactors spaced evenly between the limits appeared reasonable. Each reactor would be started from the culture developed during acclimation and would be operated sufficiently long to ensure that the data represent typical performance.

Since the majority of the organic matter entering an activated sludge reactor is nonexotic two logical questions arise concerning the input of a priority pollutant to such a reactor. The first is whether the pollutant exerts a significant effect upon the kinetics of removal of the normal organic matter. This can be determined by operating control reactors in parellel with the reactors receiving the pollutant and by analyzing them for soluble COD (SCOD) and biomass concentration. The data can then be analyzed to determine whether the pollutant exerted a significant effect upon the values of the parameters in Eq. (6) characterizing the removal of SCOD. The

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second question arises from the fact that complicated analyses are generally required to determine the concentration of a priority pollutant in a complex mixture of organic compounds. It is concerned with whether a constituent requiring less complicated analysis can be used as an indicator of the removal of the priority pollutant. Two candidates come to mind: SCOD and methylene blue active substances (MBAS). This question could be answered by collecting data on their removal on a routine basis and testing for a correlation between their concentration and the concentration of the pollutant.

The data collected during steady-state operation of the CSTR's will only reflect noninhibitory effects of the pollutant concentration upon the specific growth rate. Should information be required on inhibitory effects it will be necessary to obtain it with batch studies using cells taken from the CSTR's. The establishment of the kinetic relationships in an inhibitory region was not considered to be within the scope of this project. Thus any studies on the inhibitory effects would be exploratory in nature and no attempt would be made to fit the data to a kinetic function.

For the anaerobic studies, CSTR's without cell recycle would mimic well mixed reactors found in practice. As pointed out earlier, however, true continuous operation is difficult to attain on a small scale while feeding sewage sludge solids. Thus it is necessary to employ semicontinuous feed to well mixed reactors. Feeding can be on a once a day basis as long as the amount of material added and removed each day does not have a deleterious effect upon system performance.

The kinetics of anaerobic digestion are considerably more complicated than those of activated sludge and other aerobic processes. This is because the biomass consists of distinct, yet interacting, populations. If one is interested in the kinetics of degradation of a complex organic molecule in an anaerobic reactor then one has primary interest in the nonmethanogenic organisms since they will probably be the ones responsible for the reactions involved in that degradation. Consequently the concentration of the priority pollutant in the reactor will reflect the specific growth rate of those organisms. At the same time, one cannot ignore the fate of the methanogenic organisms because they will influence the way in which the nonmethanogenic organisms respond by serving as a sink for their metabolic products. Thus, even though the data collected on the concentration of the pollutant will speak directly only to the condition of the nonmethanogenic bacteria the SRT values chosen must be sufficiently long to allow the slower growing methanogenic organisms to maintain themselves in the population. This suggests that a lower limit on the SRT should be around 10 days. The upper limit should reflect current practice and thus can be as high as 45 days. Furthermore, whereas the objective in the aerobic studies was to obtain numerical values for the kinetic parameters in Eq. (6), the complex kinetics of anaerobic systems makes the applicability of such a simple μ:S function questionable. Consequently, the intent of the anaerobic study is to obtain data which will allow correlation of the concentration of the pollutant with the SRT. For this, three values should be sufficient.

Since it is anticipated that the extent of anaerobic degradation of many xenobiotic compounds will be small, and since many such compounds are now entering anaerobic reactors, another important question is what the effects of the compounds are upon the reactors. Thus another aspect of this work is to observe how the test compound affects the anaerobic digestion process. To accomplish this control reactors must be run at each SRT and complete performance data must be collected on them as well.

Extent of Biodegradation--Because detection of the priority pollutant is by a specific test which is dependent upon the integrity of its molecular structure, failure to detect the compound is only evidence for primary biodegradation. The question naturally arises, therefore, of the extent of biodegradation. Proof of the extent of biodegradation is quite involved and is considered to be beyond the scope of this study. Circumstantial evidence concerning it can be collected, however. One method would be through the use of randomly radiolabeled compound because then release of labeled carbon dioxide and/or methane would be indicative of cleavage of the molecule. Furthermore, consideration of the amount of radiolabeled gases produced would be suggestive of the extent to which biodegradation had occurred. Lacking radiolabeled material, indirect evidence can be obtained by supplying the compound as sole carbon and energy source and looking for the uptake of oxygen or the release of carbon dioxide and/or methane. Along this same line, a severe test for biodegradation in excess of primary would be to slowly remove all other carbon sources from the feed to a CSTR. If the compound continues to be removed and cells continue to grow then that is evidence that degradation is complete enough to allow carbon and energy to be obtained from the compound.

VOLATILIZATION--

Determination of the importance of volatilization as a removal mechanism requires estimation of the overall volumetric mass transfer coefficient, K_v (Eq. (10)). Since the aerobic reactors are aerated by bubble aeration the mass transfer coefficient must be evaluated for the same mode of transfer. This can be done by employing an aerated batch reactor which has had a known amount of the pollutant added, and then measuring the decrease in concentration within the reactor with time. Because S_e will be close to zero, the integral technique can be applied to the first-order rate expression in Eq. (10) for the determination of K_v. Because the value may be quite low for some compounds, a considerable time period may be required for the test. Thus special precautions must be used to ensure that no biodegradation occurs. This means that the test cannot be performed in the CSTR's used for the biodegradation experiments. Rather, a special sterile reactor must be employed. The question then arises as to how the mass transfer coefficient for the pollutant in that reactor may be applied to the test reactors. The most straight-forward way of doing this would be to measure the K_La value for O_2 in the special volatilization reactor and in each of the experimental CSTR's. Then the K_v values for the CSTR's can be obtained from the relationship:

$$K_{v\ CSTR} = K_{v\ VR} \frac{K_La_{\ CSTR}}{K_La_{\ VR}} \qquad (12)$$

The anaerobic reactors will be sealed and the only flow of gas will be that of the carbon dioxide and methane generated within. Because this amount of gas will be small and will be produced slowly, it is anticipated that equilibrium will be achieved between the pollutant in the liquid phase and the pollutant in the gaseous phase. Consequently the rate of mass transfer as calculated with Eq. (9) would approach zero. If the Henry's law constant for the pollutant is large, then the partial pressure in the gas phase at equilibrium is likely to be large so that some of the pollutant could be removed in the generated gas. This amount could be estimated from literature values using phase equilibrium principles. If values are not available an experiment could be devised to estimate Henry's law constant.

ADSORPTION--

As stated earlier, it does not appear realistic to try and evaluate the rate of adsorption of the pollutant onto biomass or anaerobic solids. Rather, it will be assumed that equilibrium has been achieved between the two phases and the amount removed by adsorption will be estimated from an adsorption isotherm. This means that the isotherm must be evaluated. The easiest way to evaluate the isotherm is to use standard techniques whereby the amount of material removed from solution is assumed to be on the solid and the sorptive capacity is calculated as the difference between the initial and final concentrations in solution divided by the concentration of solids present. Care must be exercised to ensure that no biodegradation occurs during the test. If acclimation has been difficult, it is unlikely that any biodegradation will occur during the course of the test if the solids have never been exposed to the test compound. Thus in many instances it would be sufficient to merely run the isotherm with unacclimated solids. If biodegradation is a concern, then the isotherm could be run in the cold to retard biodegradation. This will have a slight effect upon the isotherm values obtained, but this is likely to be small in comparison to the other experimental errors involved. Confirmation that the removal had indeed been by adsorption can be obtained by quantitatively extracting the pollutant from the solids.

When the solids contain a considerable lipid fraction, it is likely that sorption into that material will not be freely reversible. Furthermore, if the solids are brought into contact with a high concentration of priority pollutant (such as in the feed to the anaerobic reactor) the concentration of the pollutant in the soluble state in the reactor may not truly relect the amount contained within the solids. Consequently, in that case, the contribution of sorption can only be estimated by direct extraction.

BIODEGRADATION TEST PROTOCOL

The objective of the testing scheme presented here is to obtain experimental data which can be used to predict the fate of priority pollutants in conventional municipal biological wastewater treatment systems. A multilevel approach is presented for both aerobic and anaerobic systems to meet this objective. The orderly arrangement of experimental tasks and decisions required is shown in the logic flow diagrams for aerobic and anaerobic testing; Figures 1 and 2 respectively.

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In order to meet the stated objective, the three major mechanisms for removal of the priority pollutant must be evaluated and the contribution of each must be measured. This means that the often competing removal mechanisms of biodegradation, volatilization and sorption to solids need to be quantitated and assigned an order of importance.

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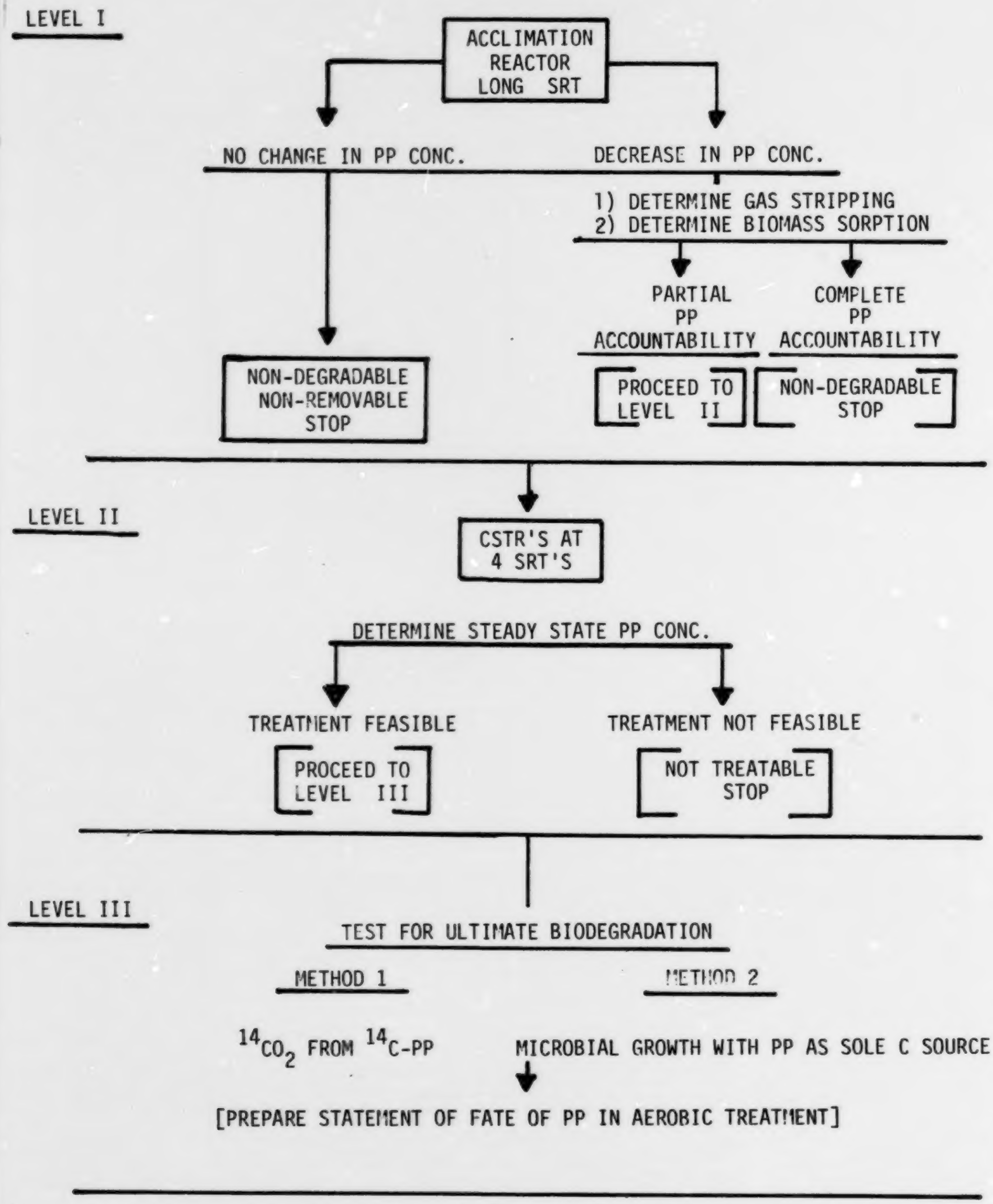
LEVEL I
ACCLIMATION REACTOR LONG SRT
NO CHANGE IN PP CONC.
DECREASE IN PP CONC.
1) DETERMINE GAS STRIPPING
2) DETERMINE BIOMASS SORPTION
PARTIAL PP ACCOUNTABILITY
COMPLETE PP ACCOUNTABILITY
NON-DEGRADABLE NON-REMOVABLE STOP
PROCEED TO LEVEL II
NON-DEGRADABLE STOP
LEVEL II
CSTR'S AT 4 SRT'S
DETERMINE STEADY STATE PP CONC.
TREATMENT FEASIBLE
TREATMENT NOT FEASIBLE
PROCEED TO LEVEL III
NOT TREATABLE STOP
LEVEL III
TEST FOR ULTIMATE BIODEGRADATION
METHOD 1
METHOD 2
$^{14}CO_2$ FROM ^{14}C-PP
MICROBIAL GROWTH WITH PP AS SOLE C SOURCE
[PREPARE STATEMENT OF FATE OF PP IN AEROBIC TREATMENT]

Figure 1 Logic flow diagram for aerobic testing.

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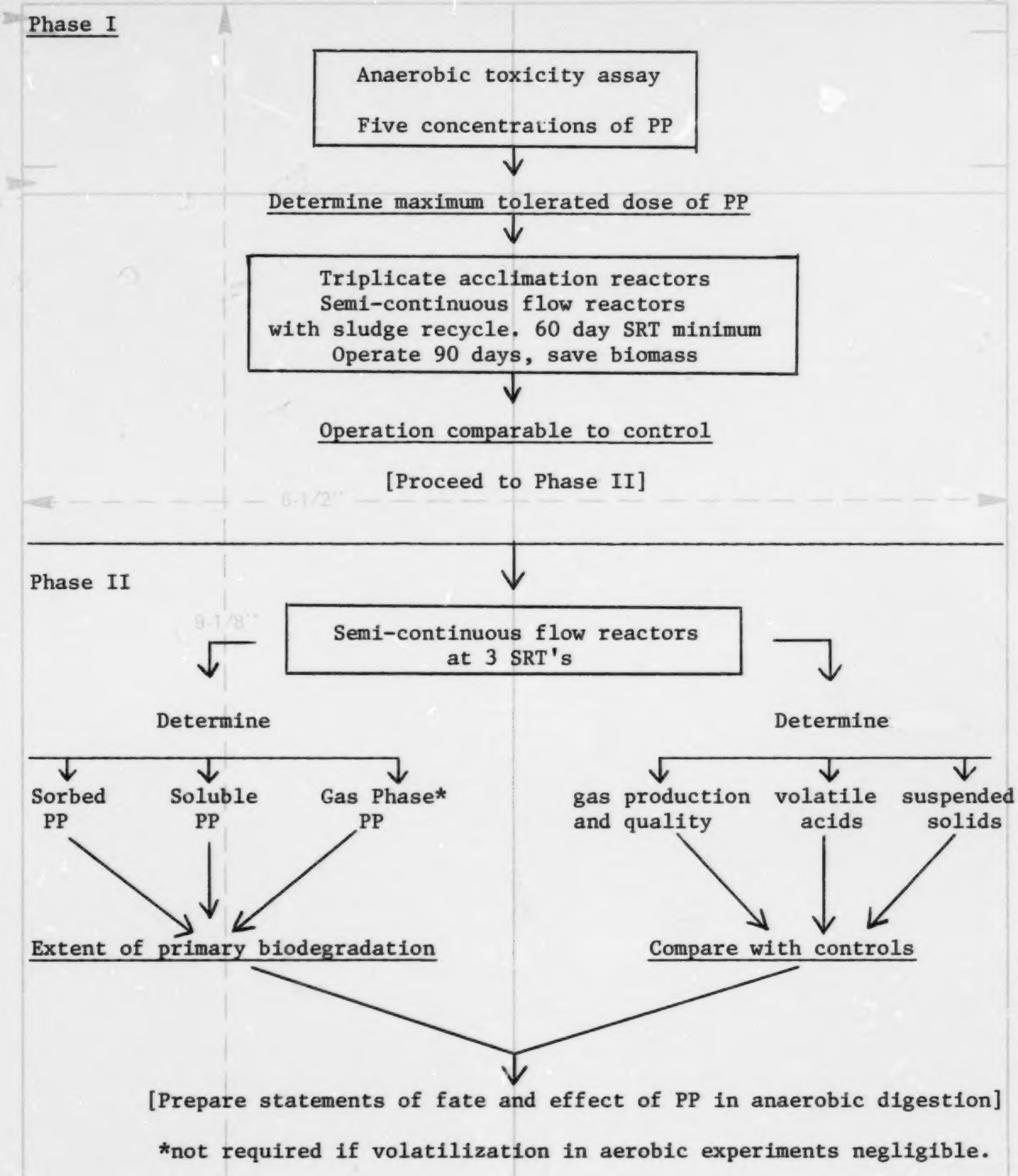
Phase I
Anaerobic toxicity assay
Five concentrations of PP
Determine maximum tolerated dose of PP
Triplicate acclimation reactors
Semi-continuous flow reactors
with sludge recycle. 60 day SRT minimum
Operate 90 days, save biomass
Operation comparable to control
[Proceed to Phase II]
Phase II
Semi-continuous flow reactors
at 3 SRT's
Determine
Sorbed PP
Soluble PP
Gas Phase* PP
Extent of primary biodegradation
Determine
gas production and quality
volatile acids
suspended solids
Compare with controls
[Prepare statements of fate and effect of PP in anaerobic digestion]
*not required if volatilization in aerobic experiments negligible.

Figure 2 Logic flow diagram for anaerobic testing.

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The aerobic testing protocol is divided into three phases as shown in Figure 1. Each phase can be said to have a unique objective. By way of summary, Phase I is used to detect removal of priority pollutant from the waste stream and to establish to what extent primary biodegradation is responsible for removal. If there is evidence of biodegradability in Phase I, the pollutant passes on to Phase II and undergoes testing at four solids retention times (SRT's) selected with a view toward "typical" field operation for municipal activated sludge systems. These data are subjected to analysis using priciples of microbial growth kinetics. Finally, Phase III testing is applied to determine whether ultimate biodegradation (mineralization) of the compound can be established.

The anaerobic testing protocol is divided into two phases as shown in Figure 2. There it will be seen that passing a compound from Phase I to Phase II is not dependent upon evidence of biodegradability in Phase I. Rather it is important that all compounds undergo Phase II testing since the protocol is intended to examine the effect of the priority pollutant upon the digestion process as well as to determine its fate in the system. Phase I testing has two distinct steps. The first is a relatively simple test to find nontoxic application levels for the priority pollutant during acclimation whereas the second is the actual acclimation. The extent of removal of the priority pollutant by biological and physical means as a function of SRT is determined in Phase II.

Aerobic Testing Protocol

Phase I--

The logic flow diagram, Figure 1, should be used as an overall guide whereas the Materials and Methods Chapter or the Results and Discussion Chapter should be consulted for the details of the experimental techniques. In general, Phase I involves the extended operation of a continuous flow fiber-wall reactor seeded with activated sludge and diverse organisms from the environment. Primary sewage effluent dosed with priority pollutant is supplied at a rate fixing the nominal hydraulic retention time at 12 hours. Although a few suspended solids are lost in the effluent, most are retained in the reactor so that the SRT is very long and the acclimated cells can accumulate along with unacclimated biomass. Priority pollutant is supplied initially at very low concentrations, but the concentration is gradually increased to 20 mg/l. The biomass formed serves as acclimated seed for Phase II reactors. Operational parameters, including the concentration of the priority pollutant are monitored. If after the stated operational period the effluent concentration is less than the influent concentration, sludge sorption and gas stripping tests are done to determine to what extent these removal mechanisms influence the priority pollutant concentration. If removal is primarily the result of biological removal rather than physical removal, Phase II and Phase III testing are initiated. If, however biological removal is insignificant, aerobic testing is discontinued and the compound is considered to be biologically recalcitrant in aerobic wastewater treatment.

Phase II--

Reactor biomass from Phase I is used to start four pairs of single-pass, completely mixed, aerated, homogeneous, continuous-flow reactors which are operated at nominal SRT's of 3, 7, 11 and 15 days. One of each pair is supplied raw settled sewage supplemented with priority pollutant according to the schedule given in the Materials and Methods Chapter. The other receives the same feed without the priority pollutant. After the reactors reach steady-state, they should be operated for a period equivalent to a minimum of three SRT's. Sampling during this time is to be done at frequent intervals since the steady-state levels of priority pollutants and other operating parameters are critical to obtaining good kinetics.

In the event that the effluent concentrations of priority pollutants are not significantly decreased by biological activity in the four reactors, it becomes apparent that biological treatment is not a viable means of destroying the pollutant and no further aerobic testing is warranted. However, if biological destruction is noted in any of the reactors, Phase III testing should be initiated.

Phase III--

One of two approaches may be used to demonstrate ultimate biodegradation (mineralization) of the priority pollutant. When randomly labelled ^{14}C-priority pollutant is available, it may be substituted for the unlabelled compound in wastewater and supplied to a concentrated biomass in a closed vessel (batch reactor) with provision for trapping CO_2 released by microbial respiration. The cells would be taken from one or more of the Phase II reactors during steady-state. A detailed method for doing this is provided in the Results and Discussion Chapter. It is recommended that the experiment be done in such a way that time-related release of CO_2 can be measured since this information can serve to provide some additional biodegradation rate data. The release of CO_2 equivalent to 50% or more of the priority pollutant carbon content should be construed to indicate substantial mineralization of the pollutant.

If radiolabelled compound is unavailable a second approach is used to infer ultimate biodegradation. It is based on the premise that the demonstration of aerobic microbial growth at the expense of a single carbon source (i.e., priority pollutant) is evidence for the conversion of at least a portion of the carbon source to CO_2 via energy-yielding respiration. The approach recommended to demonstrate the use of the priority pollutant as a carbon and energy source for microbial growth is to gradually replace all of the organic constituents in one or two Phase II reactors with priority pollutant as a carbon and energy source for microbial growth. The detailed transition to a priority pollutant feed is described in the Results and Discussion Chapter. The priority pollutant should be supplied as the sole carbon source for a minimum period of time equivalent to three SRT's. If a new steady state level of priority pollutant lower than the input concentration is observed, microbial growth can be assumed and ultimate biodegradation of the pollutant is indicated. Knowledge of the observed yield coefficient can help to fix the extent of mineralization since the observed yield usually ranges between 0.2 - 0.5.

Any priority pollutant reaching and completing Phase III testing will have provided sufficient information for the preparation of a statement of expectation regarding its fate in aerobic biological wastewater treatment. Such a statement should include as test results permit, a prediction of the minimum effluent concentration of the priority pollutant which can be expected at infinitely long SRT, as well as concentrations expected over the range of SRT's used in conventional operation of aerobic biological systems. Any disclaimers relating to potential difficulties in biological treatment which may result from toxicity or other influences of the priority pollutant on normal operation should be indicated. The relative importance of physical and biological removal mechanisms should be included. Finally the extent of biodegradation, ranging from primary to ultimate biodegradation, should be indicated.

Anaerobic Testing Protocol

Phase I--

The logic flow diagram, Figure 2, should be used as an overall guide whereas the Materials and Methods Chapter or the Results and Discussion Chapter should be consulted for the details of the experimental techniques. In general, Phase I involves two distinct experiments. First, a toxicity test is done to determine how tolerant methane production is to various input concentrations of the priority pollutant. The details of this test are given in the Materials and Methods Chapter. The highest tolerated dose of priority pollutant is determined and is used to fix the maximum input level during acclimation. In cases where the maximum tolerated level exceeds 20 mg/l, we have chosen to acclimate up to a dose of 20 mg/l. At this point, triplicate, semi-continuous flow anaerobic reactors are initiated and fed primary sewage sludge dosed with a level of pollutant equivalent to 5% to 20 mg/l or of the maximum tolerated dose if it is below 20 mg/l. The input concentration of pollutant is gradually increased to the selected maximum dose. The acclimation period should last about 90 days, during which time gas production, suspended solids, total volatile acids, alkalinity, pH and soluble priority pollutant concentration are monitored. Although Phase I experimentation is intended primarily to obtain biomass for the reactors in Phase II, it is imperative that the dosed reactors be physiologically comparable to untreated controls. Therefore control reactors are also maintained to obtain baseline data.

Phase II--

In order to inoculate Phase II reactors, acclimated sludge from the Phase I reactors is pooled and redistributed to three Phase II reactors. These reactors must undergo a transition period to bring them to steady state at their respective SRT's of 10, 20, and 40 days. The feeding schedule and sludge wasting schedule is described in the Materials and Methods Chapter. Unspiked control reactors operated at the same SRT's are also brought to steady state. All reactors should be operated for a period of time equivalent to a minimum of two SRT's. During this time regular sampling for soluble pollutant concentration, gas production, gas quality, volatile acids, suspended solids, alkalinity and pH should be done. In addition, an

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evaluation of sorption of the pollutant to sludge biomass and its removal by stripping should be done. The latter may be omitted if volatility tests in the aerobic protocol have shown that negligible gas stripping occurs.

As indicated in Figure 2 even if biodegradation does not appear to be involved in the removal of the priority pollutant, Phase II is initiated so that the effect of the priority pollutant on "normal" anaerobic digestion at 3 different SRT's can be ascertained. In the event that removal of the priority pollutant in Phase I can be partially or totally related to biodegradation, the Phase II experiment is of value in predicting the expected concentration of priority pollutant at various SRT's. Overall, the data produced can indicate to what extent the priority pollutant is treatable by an anaerobic process. Therefore, a statement of fate of the priority pollutant in anaerobic treatment which also includes the effect of the priority pollutant on anaerobic treatment can be written. Such a statement should include, as test results permit, a prediction of the minimum effluent concentration of the priority pollutant which can be expected at infinitely long SRT, as well as concentrations expected over the range of SRT's used in conventional operation of anaerobic digestion systems. Any disclaimers relating to potential difficulties in biological treatment which may result from toxicity or other influences of the pollutant on normal operation should be indicated. The relative importance of physical and biological removal mechanisms should be included.

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CHAPTER 5

MATERIALS AND METHODS

AEROBIC TESTING

Acclimation Reactor Design

A fiber-wall reactor as described in Figure 3, similar to the one described by Etzel and Kirsch (10), was used to produce an acclimated biomass. It consisted of a 12-liter Pyrex chromatography jar (Corning Glass Works), modified by inserting a 1/2 in O.D. glass nipple into its side approximately 2 inches above the bottom. A vinyl-coated wire basket, covered with nylon filter cloth with a nominal pore size of 100 μm (GAF, Industrial Products, NY-7036) was placed inside the jar. The cloth was formed into an enclosure which was sealed on the bottom and sides by stitching the seams with nylon fishing line. Four fritted glass diffusers were placed inside the enclosure. The diffusers were suspended from a plate glass lid, which was fitted with glass nipples for supporting the diffusers and influent tubing. Additional holes were provided in the lid for withdrawing samples and for removal of the exhaust aeration air. A seal, formed by 1/8 inch rubber tubing, was glued to the reactor vessel and the lid was tightly clamped down against it, preventing the escape of contaminated exhaust air into the lab. Rather, the exhaust was directed to a negative pressure ventilation system which discharged into a fume hood. Influent was pumped into the fiber-wall enclosure and flowed through the porous cloth to the effluent overflow. The position of this overflow device determined the volume of liquid in the reactor interior. The reactor feed was pumped by a Harvard Model 1200 peristaltic pump at a flow rate sufficient to give a 12 hour hydraulic residence time (HRT) in the bag interior.

To effectively retain the organisms inside the fiber-wall enclosure, the filter cloth had to be sealed. This was done by slowly adding approximately 20 liters of activated sludge, obtained from a local treatment plant, to the bag interior, allowing the strained liquid to pass out the discharge. This treatment not only sealed the filter cloth but gave the reactor a large, diverse population of organisms. After this had been done, raw sewage was pumped to the reactor to increase the number and diversity of organisms present.

To improve the chances for a successful acclimation, a batch enrichment procedure, designed to provide a PCP degrading inoculum for the acclimation reactor, was carried out. This was done by placing 50 g of garden soil into 250 ml of raw sewage to which 20 mg/l PCP and 100 mg/l Difco nutrient broth had been added. After two weeks of incubation, analysis of the culture

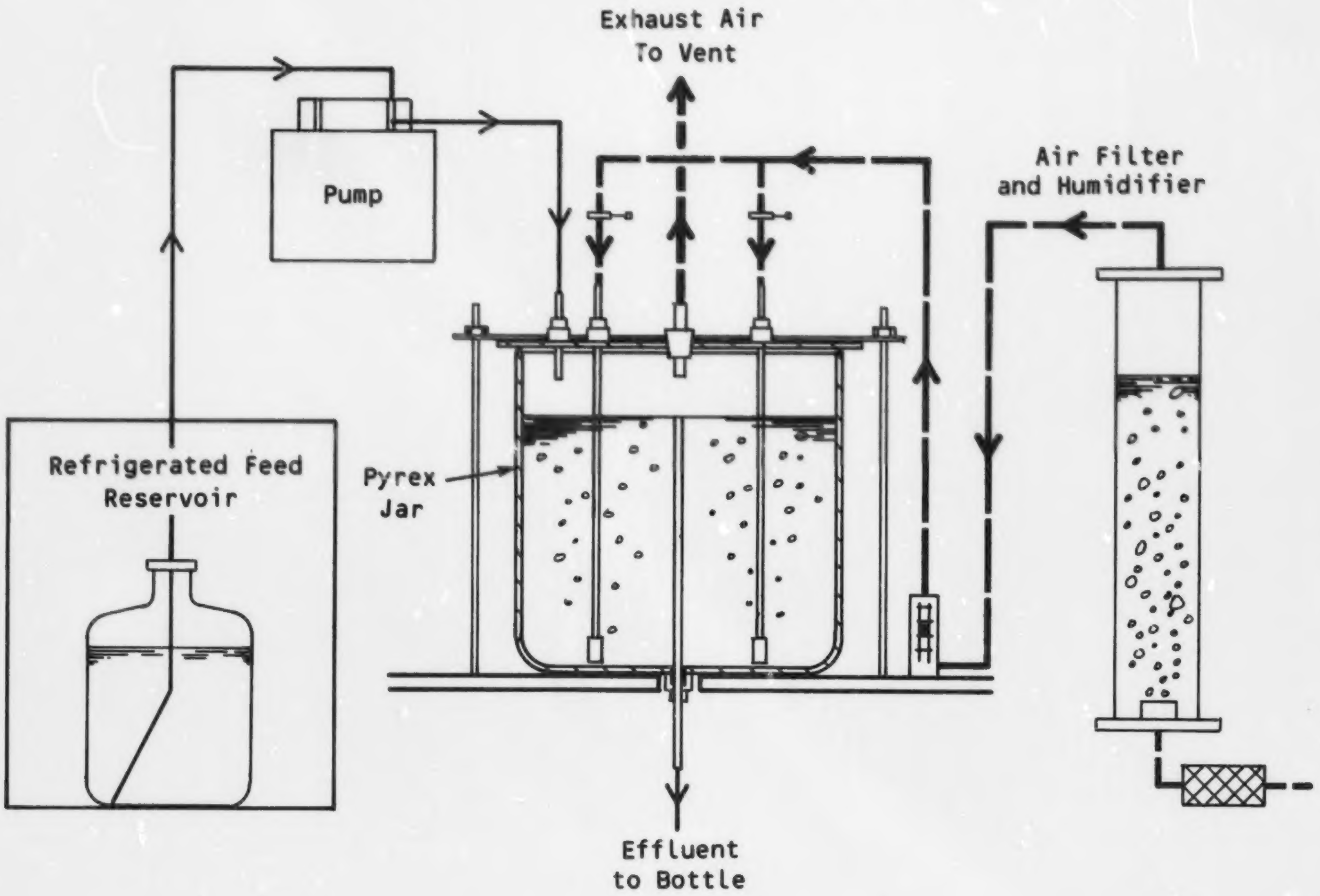
Exhaust Air
To Vent
Air Filter
and Humidifier
Pump
Refrigerated Feed
Reservoir
Pyrex
Jar
Effluent
to Bottle

Figure 3 Aerobic fiber—wall reactor as used in acclimation.

filtrate indicated that the PCP concentration had been reduced by 20 percent and thus the entire culture was added to the activated sludge biomass in the fiber-wall reactor. The acclimation process was begun by adding 1.0 mg/l PCP to the raw sewage fed to the reactors. The concentration was slowly stepped up to 20 mg/l before the biomass was considered to be fully acclimated and ready for transfer to the continuous flow test reactors.

Continuous Flow Reactor Design

The objectives of the design of the continuous flow reactors were: 1) to provide precise control over the SRT, 2) to make the reactors economical to purchase, easy to set up and easy to maintain, 3) to minimize the exposure of the test compound to the lab personnel, and 4) to reduce the likelihood of adsorption of the test compound onto the reactor interior and influent system. These were accomplished by the use of continuous stirred tank reactors (CSTRs) which did not employ cell recycle. Figure 4 shows the basic design. The reactor vessels were Pyrex chromatography jars which had a 1/2 inch O.D. glass nipple inserted in the bottom. An 8 mm glass riser tube was placed in this nipple and secured there by a Swagelok type fitting. This tube served as an effluent overflow device, the height of which determined the reactor volume. The overflow was modified later by installing 3 mm diameter glass siphon tubes at the top of each overflow tube. The siphon was so placed to require a 1/4 inch rise in reactor volume (2-5% increase in volume) before a high rate discharge of effluent occurred. The reason for this modification is reported in Chapter 6. The effluent was collected in one liter Pyrex jars. The reactor was covered by a plate glass lid, clamped onto the reactor and sealed with a rubber tubing gasket. Fritted glass diffusers were suspended from this lid into the reactor. The lid contained holes to allow for sample collection from the reactor interior and to allow for the discharge of the aeration air to the negative pressure ventiliation system.

Two sets of four reactors each were set up, one for testing PCP and one as a control. Since no cell recycle was used, the SRT would be equal to the HRT as long as the cell concentration in the reactor was uniform and equal to the concentration in the effluent. The four SRT's chosen for use in the test were 3, 7, 11 and 15 days. To simplify the influent pumping system, a common flow rate of 500 ml/day was chosen; therefore, it was necessary to choose the reactor volumes to give the desired SRT's. They were 1.5, 3.5, 5.5 and 7.5 liters. The dimensions of the individual reactors are given in Table 1. The reactors were set up on a specially built table equipped with electrical and compressed air connections and each reactor was provided with an individually controlled air supply, adjusted by a rotometer. The compressed air was filtered and humidified in a heated, automatically refilling humidifier before entering the reactors.

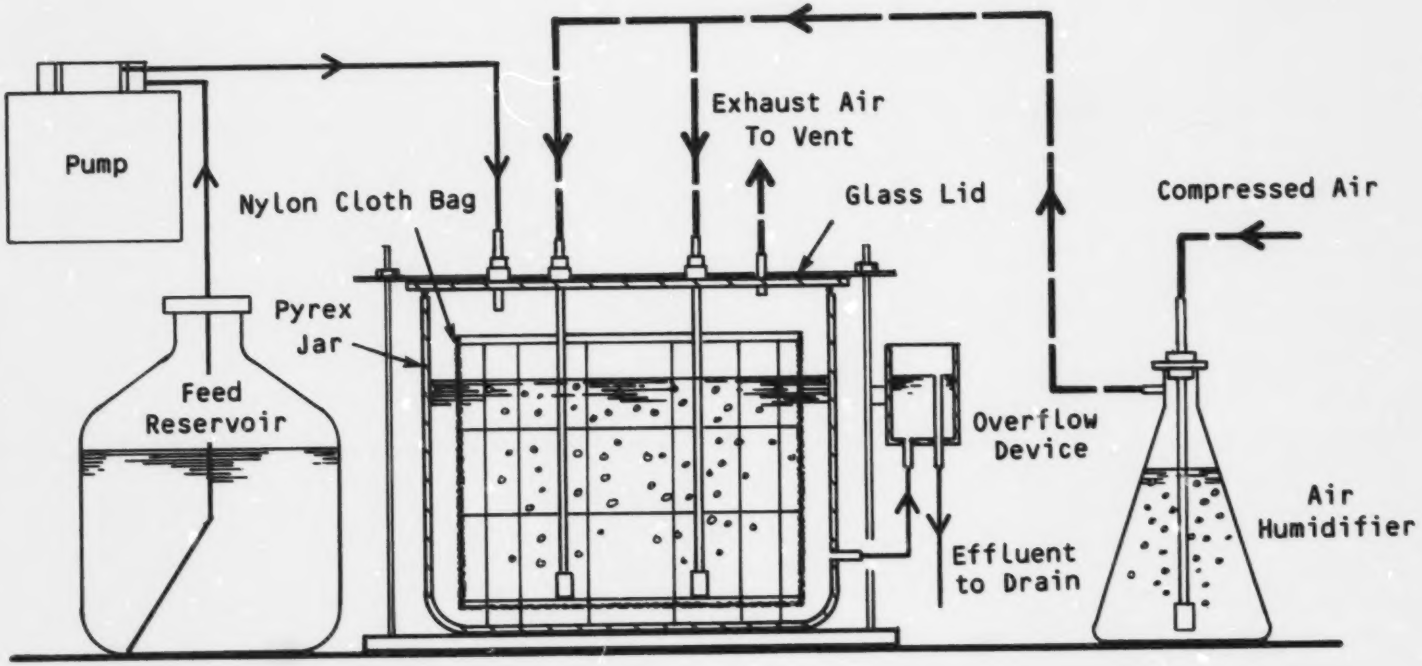
Pump
Nylon Cloth Bag
Exhaust Air
To Vent
Glass Lid
Compressed Air
Pyrex
Jar
Feed
Reservoir
Overflow
Device
Air
Humidifier
Effluent
to Drain

Figure 4 Continuous stirred tank reactor as used in aerobic Phase II testing.

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Table 1 Dimensions of Continuous Flow Reactors

Reactor Designator No.	1	2	3	4
SRT (days)	3	7	11	15
Influent Flow Rate (ml/day)	500	500	500	500
Volume (ml)	1500	3500	5500	7500
Tank Dia. (cm)	14.6	14.6	21.6	24.8
Tank Height (cm)	30.5	30.5	25.4	25.4
Water Depth (cm)	9.2	21.6	16.5	15.9
No. of Diffusers	2	2	3	4
Air Flow Rate (liter/min)	3.0	3.5	5.5	7.5

Note: The 3 day SRT reactor described here was eventually replaced with a smaller unit, 10.8 cm dia., 23 cm tall with a water depth of 15.2 cm.

The feed to each set of reactors was kept in a four liter feed reservoir, located inside a refrigerator maintained at a temperature of 6°C. The feed was pumped from these reservoirs at a rate of 500 ml/day per reactor through a Harvard Model 1200 multichannel peristaltic pump using 1/8 inch diameter silicone rubber tubing. Four channels were used for each set of reactors. Silicone tubing was used for the influent piping from the pump to the reactors. This arrangement was later modified as discussed in Chapter 6.

Feed Preparation

The feed supplied to the acclimation reactor and to the CSTR's was identical, except for the PCP concentrations in the early stages of acclimation. This feed consisted of raw sewage, which was adjusted to a constant MBAS concentration of 10 mg/l and a constant SCOD concentration of 200 mg/l. The adjusted SCOD of the test reactor feed was subsequently changed to 600 mg/l, as described in Chapter 6. A dog food extract, containing a high concentration of soluble organic matter, was used to increase the SCOD concentration when needed. It was prepared by placing approximately 900 g of dry-moist dog food (Gaines Top Choice, General Foods Corp.) in 5 liters of deionized water and autoclaving the mixture for 30 minutes. Upon cooling, the liquid supernatant was filtered, first through glass wool then through a diatomaceous earth filter. The clarified extract was frozen in aliquots for future use. It was thawed and the SCOD measured just prior to use. The sewage was settled for approximately 30 minutes and filtered through a plug of glass wool. The MBAS and SCOD concentrations were then determined. If the SCOD was less than 200 mg/l (600 mg/l) then an appropriate quantity of the dog food extract was added to it to bring the SCOD up. Similarly, the MBAS was raised to 10 mg/l, if deficient, by adding a stock solution of LAS surfactant.

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Characteristics of Raw Sewage--

The characteristics of the sewage often changed drastically, particularly during the summer months. Overall, the SCOD concentration ranged from 29 mg/l to 155 mg/l with an average of 74 mg/l; the suspended solids concentration varied from 5 mg/l to 48 mg/l with an average of 20 mg/l. The pH ranged from 7.3 to 8.1 with a mean of 7.6. MBAS concentration averaged 1.45 mg/l with a maximum of 4.2 and a minimum of 0.5 mg/l.

Characteristics of Dog Food Extract--

The dog food extract contained approximately 60 g/liter of soluble COD. The dog food from which the extract was made contained almost 20% protein, of which approximately 20% was nitrogen. From this it was assumed that it would contain ample organic nitrogen to provide a well balanced feed. The actual amount of nitrogen in the dog food extract was not determined until after phase II had been completed. That analysis yielded the information in Table 2. The nitrogen requirement of the systems can be calculated from the net sludge production from the organics in the feed. The COD in the feed during most of phase II testing was changed to 600 mg/l, rather than 200 mg/l to permit the reactors to operate at higher suspended solids concentration. The reason for this change is described more fully in Chapter 6. Assuming that 12% of the cell mass is nitrogen, and using an observed growth yield of 0.3, the amount of nitrogen required for utilization of 600 mg/l of COD is 22 mg/l. Since the average SCOD of the sewage was 74 mg/l, sufficient dog food extract was added to provide 526 mg/l SCOD. This means that 8 mg/l of nitrogen would be added as well so that 14 mg/l must be provided by the sewage. In order to avoid possible deficiency of nitrogen, the feed recipe was supplemented with 20 mg/l of urea nitrogen. As can be seen in Table 2, the dog food extract contained ample phosphorus.

Table 2 Dog Food Extract Nutrient Analysis

	Approx. Concentration (mg/l)
COD	55,000
NH_4^+-N	40
Organic N	800
Nitrate	6
Nitrite	0
Ortho Phosphate	1,800

Preparation of PCP Stock Solution--

The pentachlorophenol used to prepare the feed was reagent grade, 99+% purity (Aldrich Chem. Co., Milwaukee, Wis.), boiling point 188-191°C. It was made into the sodium salt by the following procedure:

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1) 10 grams PCP were dissolved in approximately 300 ml of 1.0 N NaOH.
2) Concentrated H_2SO_4 was slowly added to bring the pH down to ~6.0. Precipitates formed from addition of acid were allowed to redissolve before more acid was added.
3) Deionized water was added to increase the volume to 1.0 liter.

Sufficient quantities of this stock solution were added to the test reactor feed to yield a concentration of 20 mg/l as PCP. Analysis of the sewage detected no background PCP.

Preparation of MBAS Stock Solution--

The surfactant added to the feed to maintain an MBAS concentration of 10 mg/l was a commercial preparation containing a variety of isomers, the majority of which were C_{10}, C_{11}, and C_{12} homologs, though a significant amount of C_{13} material was present also. The material, CONOCO C-550 Slurry, (Conoco Chemical Co., Ponca City, Oklahoma) was a thick, syrup-like slurry which was carefully mixed before being diluted with deionized water. The material was diluted on a volume basis and the final stock solution was analyzed for MBAS so that the amount required for addition could be calculated accurately.

Sampling Schedule and Sample Handling

During acclimation, the reactor was tested occasionally for effluent SCOD, PCP, and MBAS. During phase II testing the schedule shown in Table 3 was adhered to.

The samples for SCOD, MBAS and PCP analysis were removed directly from the reactor interior and were immediately centrifuged at 10,000 rpm for 10 min. The centrate required for SCOD analysis was added directly to a prepared COD flask. The aliquot for MBAS analysis was processed immediately according to the protocol described later. The volume required for PCP analysis was placed in a 20 ml glass snap top vial and refrigerated until the analysis was done, (usually 2-24 hours). The solids removed from the sample were used to measure the suspended solids concentration.

Table 3 Sampling Schedule for Phase II Testing

Item	Frequency
Effluent Soluble COD	3 per week
Effluent PCP	3 per week (same day as SCOD)
Effluent MBAS	2 per week
Reactor Suspended Solids	2 per week
pH	1 per week

Analytical Techniques

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Chemical Oxygen Demand--

The chemical oxygen demand of the soluble material in the sewage and the dog food extract was determined by the dichromate reflux method as described in Standard Methods for the Examination of Water and Wastewater. (11) One modification to the procedure was the use of 11 g of Ag_2SO_4 per 9.0 lb bottle of concentrated H_2SO_4 rather than 22 g. The insoluble fraction of the sample was removed by filtration through 0.45 µm pore-size membrane filter, pre-washed three times with 50 ml of deionized water.

The soluble COD concentrations in the CSTR and acclimation reactors were measured by the dilute dichromate reflux method described in Standard Methods. (11) Each week, a sufficient number of flasks was prepared to store the entire week's samples, which were then refluxed and titrated together. The flasks were prepared by adding the dichromate and mercuric sulfate to each flask. The acid was added just before refluxing. Duplicate blanks and standardization flasks were used for greater accuracy. The reactor SCOD concentrations were measured singly. An error analysis of this technique gave a standard deviation of 0.67 mg/l and a coefficient of variation of 3% at 22.5 mg/l.

PCP Analysis--

Two methods of PCP analysis were used in this study. A modification of the 4-amino-antipyrine method, as described by Etzel and Kirsh (10), was used during the acclimation phase and during the startup of the CSTRs. A more sensitive G-C analysis was developed and was used for the remainder of the work. The modification of the wet chemical technique, which was designed for samples with less than 5 mg/l PCP, is as follows:

1) Add 100 ml of solids-free sample or a sample diluted to yield a PCP concentration of approximately 1 mg/l to a 250 ml separatory funnel.
2) Prepare 100 ml of a 1.0 mg/l PCP standard in D. I. water and add to a separatory funnel.
3) Add 100 ml of solids-free control reactor effluent to a separatory funnel. This will serve as a reagent blank.
4) Adjust the pH in each separatory funnel to 3;lower with 6 N HCl.
5) Add 25 ml of hexane.
6) Shake 60 times, venting occasionally.
7) Discard the aqueous layer.
8) Add 25 ml of 0.1 N NaOH to the hexane.
9) Shake 60 times.
10) Remove the NaOH layer to a clean separatory funnel.
11) Discard the hexane.
12) Adjust the pH of the NaOH layer to 6.0 with 6 N HCl and 1 N NaOH.
13) Add 25 ml 0.5 M phosphate buffer, pH 6.0.

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14) In each separatory funnel, swiftly perform the following sequence:

   a) Add 1.0 ml 0.4% 4-amino-antipyrene and mix.

   b) Add 1.0 ml 10% potassium ferricyanide and mix.

   c) Add 10 ml xylene.

   d) Shake 40 time, allow thorough separation.

   e) Remove the water layer and deposit the xylene layer in a marked test tube.

   f) Repeat the steps from a) to e) with a new sample.

15) Read the absorbance at 575 nm in a spectrophotometer after zeroing the instrument with the reagent blank.

16) PCP concentration is calculated by:

$$\frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{conc. of standard} = \text{mg/l PCP}$$

With a 100 ml sample volume this technique has a sensitivity of 0.05 mg/l (50 μg/l) and is quantitative to 0.10 mg/l (100 μg/l).

A shortened form of the procedure was used to estimate the concentration of PCP in samples of unknown concentration. This was extremely helpful in making the proper dilutions for the G-C analysis. It proceeded as follows:

1) Filter 30 ml of sample into a 100 ml vial.

2) Add 10 ml of 2 N phosphate buffer (pH 6).

3) Add 1 ml 0.4% 4-amino-antipyrene; mix.

4) Add 1 ml 10% potassium ferricyanide; mix.

5) Add 5 ml xylene, shake.

6) Estimate the concentration by a comparison with standards put through the same procedure.

A gas chromatographic analysis for PCP was developed which involved extraction of the PCP from a clarified, acidified sample, derivatization of the PCP to PCP-chloroacetate and cleanup of the solvent phase sample. The procedure, as it was used for PCP analysis in the aerobic systems was as follows:

Reagents: distilled benzene (Mallinckrodt Inc.)
2% chloroacetic anhydride in benzene (Eastman Kodak Co.)
0.125 N NaOH (reagent grade, Mallinckrodt Inc.)
1.5% Na_2CO_3 (reagent trade, Mallinckrodt Inc.)
Na_2SO_4 solid (reagent grade, Fisher Scientific)

Procedure: 1) Centrifuge samples at 10,000 rpm for 10 minutes.

2) Add 10 ml centrifuged effluent to 125 ml separatory funnel. Pre-extract or bake funnels at 350°C before use.

3) Add 40 drops of concentrated H_2SO_4.

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4) Add 10 ml distilled benzene.

5) Shake 100 times, allow thorough separation.

6) Discard the liquid phase, retaining the benzene.

7) Add 20 ml of 0.125 N NaOH.

8) Shake 60 times and allow thorough separation.

9) Add 10 ml 2% chloroacetic anhydride-benzene solution by draining down the wall of the separatory funnel to minimize contact of anhydride with water.

10) Shake vigorously for 2-3 seconds only.

11) Discard NaOH layer as soon as separation occurs.

12) Add 20 ml 1.5% Na_2CO_3.

13) Shake 10 seconds and allow thorough separation.

14) Discard Na_2CO_3 layer.

15) Add approximately 1/2 spatula of Na_2SO_4 to clean, baked sample vial.

16) Flush delivery tube of separatory funnel with the first 4-5 ml benzene.

17) Add next 10 ml benzene to the sample vial containing the Na_2SO_4 and cap tightly.

18) Analyze by G-C.

GC Operating Conditions

Injector temp.	190°C
Detector temp. (ECD)	300°C
Column temp.	180°C
Carrier gas flow rate (N_2)	40 ml/min

Column - 1% SP-1240 DA. 100/120
SUPELCOPORT, G-1620,
50 cm x 3.2 mm SS
elution time 3.2 min.

This analysis was determined to yield a standard error of ± 5% at 100 μg/l PCP.

The instrument used for the GC analysis was a Varian model 2700, equipped with microprocessor control and automatic peak area integration. In general, the PCP peak was well defined and isolated from most interfering peaks. Glassware contamination was found to be a major cause of interference which was solved by baking the separatory funnels at 350°C, by pre-extracting the funnels with benzene or by both. The maximum sensitivity of the instrument appeared to be approximately 5 μg/l and the response was linear to several hundred micrograms per liter. However the extraction procedure became nonlinear above 250 μg/l, restricting the useful range of the analysis unless dilution was applied.

Figure 5 shows a typical chromatogram recorded at a chart speed of 1.0 cm/min.

MBAS Analysis--

The determination of the concentration of methylene blue active substances (MBAS) was done by the colorimetric method given in Standard Methods (11). The only change in the procedure was the use of an internal standard rather than a standard curve. It was found that this gave more consistent results due to day to day variations in reagent strength and sample composition. The MBAS concentration was determined by the following:

1) A sample was filtered and divided into two aliquots.
2) One aliquot was tested according to Standard Methods (11).
3) The second aliquot was spiked with 100 μg of commercial LAS preparation and tested.
4) A reagent blank was also taken through the procedure.
5) The concentration was calculated by:

$$\text{MBAS in Sample } (\mu g) = 100\ (\mu g)\ \frac{\text{O.D. of Sample}}{\text{O.D. of Spike}}$$

where O.D. of spike = Optical Density of Spiked Sample - Optical Density of Unspiked Sample

$$\text{and MBAS Conc. (mg/l)} = \frac{\mu g \text{ MBAS}}{\text{ml Sample}}$$

Routinely, single tests for reactor MBAS concentration were run. The MBAS concentration in the feed was run in duplicate to ensure higher accuracy in the control of the influent levels of MBAS.

An error analysis of this procedure revealed a coefficient of variation of about 7% at a concentration of 1.4 mg/l.

Suspended Solids Analysis--

This analysis was done in accordance with the total suspended matter test given in Standard Methods. (11) Rather than using the glass fiber filter as specified however, a Millipore brand membrane filter, with nominal pore size of 0.45 μm, was used. Both weighings of the filter were done with the filter in a lightweight aluminum dish. The samples, usually 40 ml each, were often spun down in a centrifuge at 10,000 rpm for 10 minutes before being filtered. This greatly improved the filter-ability of the sample.

The error analysis of this procedure showed a standard deviation of 8.8 mg/l and a coefficient of variation of 6.6% at 133 mg/l.

pH Measurement--

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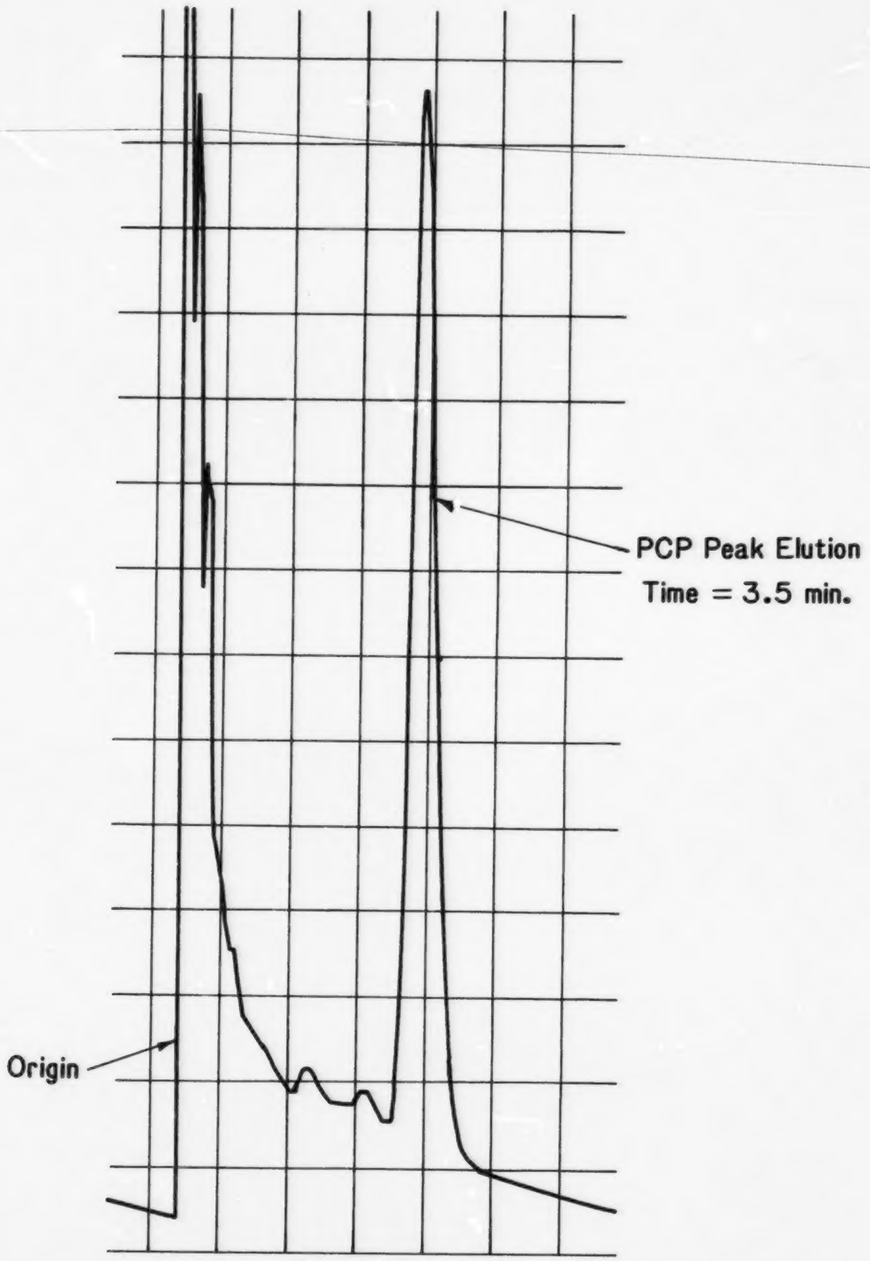

Figure 5 Typical GC Chromatogram for PCP Analysis

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The pH of all samples was measured by the glass electrode method using a Fisher Accumet Model 600 pH meter equipped with a standard combination electrode. The electrode was standardized by immersion into a pH 7.0 buffer just prior to use. Temperature effects were corrected for on the pH meter itself.

ANAEROBIC TESTING

Reactor Design

The objectives of the anaerobic reactor design were: (a) to provide for precise control over the anaerobic digestion process through maintenance of constant hydraulic residence time (HRT), solids residence time (SRT), and organic loading rate; (b) to have sufficient reactor volume to permit sampling without altering system performance; (c) to maintain strict anaerobic conditions within the reactor and gas collection system; (d) to provide for accurate measurement of the quality and quantity of gas produced; and (e) to make the system economical and simple to operate.

A continuous stirred tank reactor (CSTR) would be the logical choice if controlling the biological process were the only objective of reactor design. However, practical considerations lead to the conclusion that a CSTR would not meet the other criteria given above. Among the problems would be: the pumping of raw sewage sludge at low flow rates, refrigeration of feed, continuous removal of effluent, measurement of gas production, and collection of representative samples. Furthermore, the cost and complexity of such a system would be magnified by the number of reactors required for the study.

A semicontinuous stirred tank reactor (SCSTR), with a floating cylinder for gas collection and measurement, was chosen as the design best able to meet all of the objectives listed--provided three conditions could be met: (a) the reactor feed was constant in composition; (b) the reactors were completely mixed; and (c) the HRT was long enough to minimize changes in the reactor contents caused during the feeding process. These conditions were met throughout the study, as described below.

The operation of the SCSTR's involved the removal of digesting sludge at the same time each day, followed by the immediate addition of an equal volume of raw sludge. Since SRT was long (exceeding 10 days) the performance of an SCSTR approximated that of a CSTR operated at the same residence time. It should be noted that many full scale anaerobic sludge digesters are operated in a semicontinuous manner, being fed only once per day or per shift.

Identical equipment was used for both Phase I and Phase II testing. The reactors and gas collectors were located in a constant temperature room which was maintained at 36-37°C. Each reactor had a liquid volume of 3000 ml and was made from a 4000 ml Pyrex aspirator bottle (Corning No. 1220) which was sealed with a number 10 rubber stopper and silicon bathroom sealer. (Figure 6) Two glass tubes were run through the rubber stopper to permit daily feeding and continuous gas removal. The feed tube (12 mm diameter) extended below the liquid level in the reactor, thereby minimizing the introduction of oxygen and the loss of gas during feeding. Sludge was removed for analysis and wastage through the port at the bottom of the aspirator bottle. Tygon

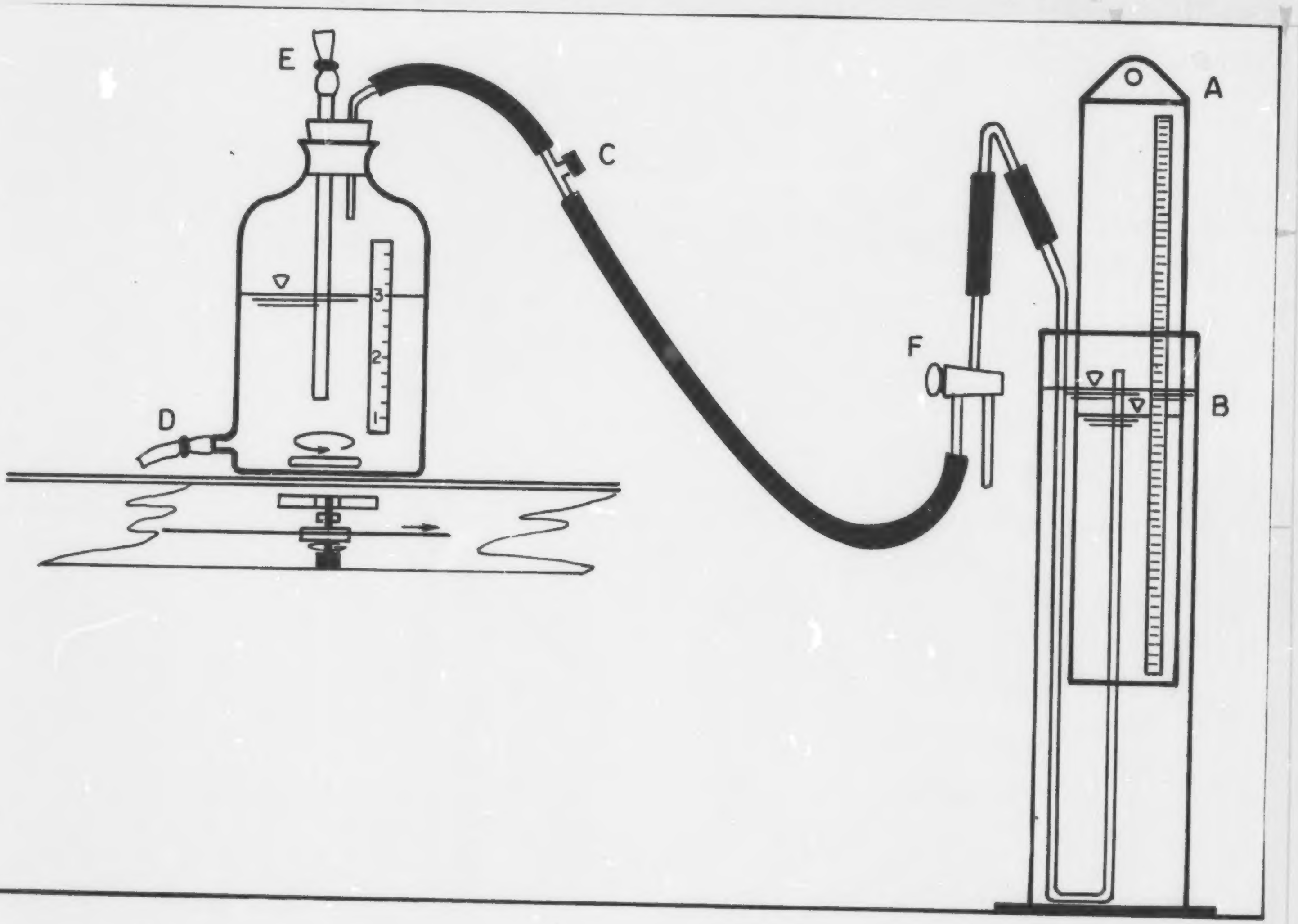

Figure 6 Semicontinuous stirred tank reactor as used in anaerobic testing.

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tubing was attached to that port, as well as to the feed tube, and both were closed with small hose clamps. The contents of the reactors were continually, and completely, mixed by a four inch, teflon-coated magnetic stirring bar which was driven by a rotating magnet beneath the reactor. The six-position, belt-driven stirring tables, upon which the reactors were placed, were designed and built at Purdue University for use in the constant temperature room.

Gas from each of the reactors was collected for daily measurement using an inverted cylinder which was floated in a saturated solution of sodium chloride. (Figure 6) The gas collectors were also made in the Civil Engineering Machine Shop, and the material of construction was a clear acrylic plastic. The gas line from the reactor to the gas collectors was made of glass tubing and butyl rubber tubing (1/2" OC x 1/4" ID; U.S. Rubber Co., No. C4-4504578C) to minimize the diffusion of gas through the walls. A three-way, double oblique bore, ground glass stopcock (Corning No. 7380) was placed in the line so that the gas collector could be vented, repaired, or replaced without exposing the reactor to oxygen. A plastic "T" was also placed in the line, and was fitted with a flexible rubber septum for use in gas sampling. The gas collector design permitted the addition or removal of sludge without risk of introducing oxygen to the reactor since gas could pass from the reactor to the collector and back again in response to changes in the liquid volume in the reactor. The entire system worked under a low, but positive, pressure from the weight of the floating cylinder. Therefore, the potential for leaks was minimized and the opportunity for oxygen to enter the system was reduced.

The daily gas production was determined by lifting the floating cylinder until its inside and outside liquid levels were the same (see Table 4); this had the effect of correcting the gas volume reading to atmospheric pressure. A tape, calibrated in tenths of liters, was attached to the floating cylinder and was waterproofed with an acrylic spray coating. Readings were made by approximating the gas volume to the nearest hundredth of a liter. Evaporation losses were made up daily with deionized water following the venting of the collectors. Two sizes of gas collectors were used, 2.25 liters and 4.20 liters, with the larger collectors being used with reactors operated at shorter retention times.

The gas collecting device permitted accurate determination of the volume of gas produced each day, because the volume was measured directly and at atmospheric pressure.

Table 4 Steps in Feeding Anaerobic Reactors (Refer to Figure 6)

---

(1). Measure gas volume by raising the floating cylinder (A) until liquid levels are the same (B) and read to nearest 0.01 liter using calibrated tape.

(2). Sample gas using the septum (C).

(3). Remove sludge for wastage and analysis (D).

(4). Feed raw sewage sludge to the reactor (E).

"continued,"

Table 4 (continued)

(5). Vent the gas collector by turning the stopcock (F).

(6). Add deionized water to replace evaporation losses from the gas collector and reset the stopcock.

Conversion to standard conditions was a simple matter of correcting for temperature and pressure:

$$\text{standard gas volume} = \frac{(\text{volume})\ (\text{pressure})\ (273.0)}{(\text{temperature})\ (760.0)} \qquad (13)$$

where:

volume = measured volume to nearest 0.01 liter
pressure = measured atmospheric pressure in mm Mercury
temperature = temperature of digester room in degrees Kelvin

Feed Characterization and Storage

Primary sewage sludge was obtained from the West Lafayette, Indiana wastewater treatment plant for use as the anaerobic reactor feed. This source was chosen several times during the study; however, enough sludge was collected each time to provide a feed with constant characteristics during an entire experimental phase. This was done to eliminate gross changes in the reactor influent thereby simplifying the interpretation of the experimental results. When necessary, the sludge was screened to remove large objects which might have plugged the feed tubes or sample ports of the reactors. It was then poured into several 40 gallon plastic trash cans (the number depending upon the amount collected) and mixed with 1/20 horsepower electric mixers (Lightnin' model 10X) while sludge from the several cans was intermixed to ensure uniformity within the batch. Aliquots of about 500 ml were then placed into plastic bags for freezing (Dow, Ziploc, 1 qt) so that a small quantity could be thawed for use each day. Sufficient freezer space was available for the storage of over 1000 bags.

The characterisitics of the feed were determined from samples of the thawed sludge. Analyses were performed several times during the period of use of each batch. Average values for each batch are presented in Table 5 along with the experimental phases(s) during which each batch was used.

Table 5 Feed Sludge Characteristics

Date of Collection	Period of Use		TS mg/l	TVS mg/l	TSS mg/l	VSS mg/l	TCOD mg/l
3/6/69	Start-up	$\bar{X}$ =	39533	24561	39335	24359	35040
		s =	686	687	-----	-----	1420
6/5/79	*PCP-Ia	$\bar{X}$ =	22415	16650	19866	15106	28413
		s =	880	451	1446	921	2577

Table 5 (continued)

Date of Collection	Period of Use		TS mg/l	TVS mg/l	TSS mg/l	VSS mg/l	TCOD mg/l
1/7/80	PCP-Ib	$\bar{X}$ =	22803	15345	21532	14821	29551
		s =	1491	1087	914	650	3442
7/5/80	PCP-II	$\bar{X}$ =	36235	22155	34383	20964	35331
	PCP-III	s =	5692	2756	5382	2726	4277

*Phase I experimentation on PCP was performed twice. The letters "a" and "b" denote those two periods.

Operation and Sampling Procedures During Phase I

One of the objectives of this study was to provide an acclimated biomass for subsequent Phase II testing. Therefore, triplicate anaerobic reactors were used for the Phase I testing with three additional reactors serving as controls. A very long SRT was chosen for the acclimation period in order to maximize the opportunity for the desired population to develop. An HRT of about 30 days was chosen for convenience of operation as well as for the organic loading rate it provided. Thus recycle of effluent suspended solids was necessary to achieve an SRT in excess of 30 days. Mixing limitations dictated, in part, the SRT which could be used, since complete mixing was not achievable at high suspended solids concentrations (above 30,000 mg/l) without a major change in the anaerobic reactor design. Since it was decided that an SRT in excess of 60 days would allow adequate acclimation, at least half of the effluent suspended solids had to be centrifuged and returned to the reactor.

As a matter of convenience, the operational procedures used for sampling, feeding, and recycling solids were not the same every day. Since the influent conditions were to be changing throughout the acclimation period due to increasing concentrations of the test compound in the feed, attainment of steady-state conditions within the reactors was impossible. Given this unsteady-state, and the long hydraulic and solids retention times employed, small variations in the daily routine of sludge removal and feeding were not critical to reactor performance. Consequently, the operational procedure was simplified by removing sludge from the reactors three times a week, even though raw sludge was added every day. On the days when sludge was not removed, the reactor volume was allowed to increase by the amount of raw sludge added that day. When sludge was removed, the volume taken from the reactor was greater than the volume fed that day. Although the sludge volume in the reactor varied by 3-5 percent on a day-to-day basis, the average volume was maintained at 3000 ml and the daily variations were small enough to have no detectable effect on reactor performance. Table 6 summarizes the events occurring each day with regard to routine reactor operation.

Every Monday, Wednesday, and Friday, five centrifuge tubes were filled with 40 ml each of digesting sludge removed from the reactor. (On Mondays only, an extra 100 ml of sludge was discarded). The sludge was then centrifuged for 15 minutes at 18,400xG in a Sorvall model SS-1 Superspeed Angle Centrifuge. After separation, the liquid fraction was removed for analysis, leaving a pellet of solids in the bottom of each tube. One of these pellets was used in the determination of suspended solids ocncentration, but the other four, representing solids from 160 ml of sludge, were placed into a 400 ml beaker and were mixed with raw feed sludge. The same procedure was followed for sludge taken from all of the reactors; however, the solids pellets from the same group of reactors were placed into a single beaker. For example, four pellets from each of the three PCP reactors were combined. A spatula was used to break apart the pellets and homogenize them with the raw sludge. The total volume of the homogenate was then increased to 350 ml with additional raw sludge. This mixture was then placed into a stoppered bottle, labeled according to the reactor group (for example, "PCP SLUDGE"), and refrigerated. On the following day (which would be Tuesday, Thursday, or Saturday) this mixture was fed to the appropriate group of reactors, thereby recycling solids. Although only 100 ml of sludge was fed to a reactor each day, it was necessary to make 350 ml of the mixture to provide for that which was lost during transfer steps. The fact that recycled solids were intermixed between the reactors of a particular group was not critical to their performance, since the reactors were operated under identical conditions; however, it is possible that some advantage was realized from the constant cross-seeding which resulted. Because of the time required for centrifugation and homogenization, recycling solids on the day of their removal from the reactors was impossible--if operational requirements of an SCSTR were to be met. On the days when sludge solids were not incorporated

Table 6 Operational Procedures for Anaerobic Reactors During Phase I (Acclimation)

	SUN	MON	TUE	WED	THU	FRI	SAT
Volume of digesting sludge removed (ml)	---	300	---	200	---	200	---
Volume of raw sludge added (ml)	100	100	83	100	83	100	83
Volume of solids recycled (ml)	---	---	17	---	17	---	17
Equivalent sludge volume of recycled solids (ml)	---	---	137	---	137	---	137

Reactor volume = 3000 ml; HRT = 32.4 days; SRT = 72.8 days into feed (Monday, Wednesday, Friday, and Sunday), 100 ml of raw sludge was added to each reactor.

Because of the operational procedures which were followed during acclimation, the actual hydraulic and solids retention times had to be calculated. Determination of the SRT involves a careful accounting of the sludge wastage rate, which is best done on a weekly basis. Each week, 700 ml of digesting sludge was removed from each reactor. Table 6) Suspended solids from 480 ml of this sludge (160 ml x 3 days) were mixed with raw sludge to make reactor feed; the other 220 ml were discarded or used for analysis. As mentioned earlier, only 300 of the 350 ml of feed containing recycle solids was actually added to the reactors; thus, 1/7 of the recycle solids were lost each week. The SRT can be calculated as follows:

$$\mathrm{SRT} = \frac{(3000\ \mathrm{ml})\ (7\ \mathrm{days})}{(220\ \mathrm{ml}) + (480\ \mathrm{ml}/7)} = 72.8\ \mathrm{days} \qquad (14)$$

The HRT is determined by correcting for the volume of recycle solids in the feed, since they effectively decrease the "flow" through the reactor. The volume of the solids pellet after centrifugation was approximately 5 ml. Since four pellets from each of three reactors were mixed with raw sludge, 60 of the final 350 ml were recycle solids; or 17 percent of the feed volume on three days a week was due to recycling. The HRT can then be calculated on a weekly basis:

$$\mathrm{HRT} = \frac{(3000\ \mathrm{ml})\ (7\ \mathrm{days})}{(400\ \mathrm{ml}) + (0.83)\ (100\ \mathrm{ml})\ (3)} = 32.4\ \mathrm{days} \qquad (15)$$

These values for the hydraulic and solids retention times were considered to be adequate to meet the objective of the acclimation period.

PCP was added to the appropriate bottle of feed immediately prior to feeding the reactors. The amount which was added to the 350 ml of feed sludge was determined from the acclimation schedule, which outlined the step-increases in influent concentration of the compound as a function of time. The acclimation schedules for PCP are presented and discussed in Chapter 6. In order to minimize dilution of the feed, the solution of PCP used to spike the feed sludge had a concentration of 1 mg/ml.

Even on days when recycle solids were not to be incorporated in the feed, 350 ml of raw sludge was placed in a stoppered bottle and refrigerated overnight before adding the PCP and feeding the reactors. Each day then, (a) sludge was taken from the refrigerator, spiked with test compound, and fed to the reactors; (b) thawed sludge was measured out and, on the appropriate days, mixed with recycle solids before being placed in the refrigerator as the next day's feed; and (c) frozen sludge was set out to thaw overnight.

Typical operating parameters for anaerobic digestion include: total suspended solids (TSS), volatile suspended solids (VSS), total volatile acids (TVA), total alkalinity (TA), daily gas production, and pH. Each of these analyses was performed three times each week--on the days when sludge was removed from the reactors. Analysis for the test compound in the reactor effluent was performed with a similar frequency whenever possible. Often, samples had to be stored for a few days prior to analysis. Following centrifugation, the liquid fraction (centrate) was transferred to 25 ml glass vials for storage at 4°C.

Operation and Sampling Procedures During Phase II

The objectives of Phase II testing were to determine (a) any deleterious effects of the test compounds upon the anaerobic digestion process in wastewater treatment and (b) the kinetics of degradation of the test compounds. Acclimated sludge developed in Phase I for each of the test compounds was used as the starting sludge for testing at three different hydraulic (solids) retention times. Data were collected during the transition between acclimation and operation at the different retention times, as well as during the subsequent steady-state operation.

The reactors and gas collectors used in Phase II were the same as those used in acclimation. The acclimation process resulted in about 9 liters of sludge for each test compound and for the controls. The sludge from one group of reactors (for example, sludge from the three PCP reactors) was pooled in a 10 liter glass carboy under a 70 percent nitrogen and 30 percent carbon dioxide atmosphere to ensure that the starting sludge for Phase II was the same in each of the three reactors. The sludge was then returned to the reactors.

Because no solids recycle was performed during Phase II, and because the reactors were completely mixed, the SRT was controlled by, and equal to, the HRT. For PCP testing, these values were 10, 20, and 40 days. The proper retention time was imposed upon the system by maintaining the liquid volume in the reactor at 3000 ml while varying the amount of sludge removed from and fed to the reactors daily. A control reactor was operated at each of the retention times to provide a basis for determining the effects of the test compound on the process. The transition from Phase I to the three different retention times of Phase II was to minimize "shocking" the reactors with decreasing retention times and increasing organic loadings. The transition schedule which was used is presented and discussed in Chapter 6.

A constant feed was provided for the entire experimental phase. Each day, thawed sludge was measured into a bottle to which the test compound was added immediately before feeding. PCP was fed at 5 mg/l rather than 20 mg/l as planned because of its inhibitory properties. (See methanogenic toxicity assay in Chapter 6). The control reactors were fed the raw sludge without PCP. Because the same sludge was fed to all reactors, the organic and test compound loading rates (mass per day per reactor volume) varied with the retention time.

The parameters measured during Phase I were also measured during Phase II to characterize reactor performance: TSS, VSS, TVA, TA,pH, and daily gas production. In addition, during the steady-state operating period, the effluent total chemical oxygen demand (TCOD) and the composition of reactor off-gases were determined. The frequency of each analysis during the transition and steady-state periods was at least three times a week. When necessary, samples of centrifuged effluent were stored at 4°C.

Methanogenic Toxicity Assay

In order to provide preliminary information which would be helpful in planning Phase I testing as well as in the transition to Phase II, a short-term, batch culture bioassay was used for evaluation of the effect of PCP upon the methanogenic organisms in digesting sewage sludge. The procedure of Owen, et al. (12) was used with some minor modifications. By comparing the apparent rate of methanogenic activity in "spiked" versus control batch cultures one may determine the degree of inhibition caused by the test compound. Furthermore, by using a wide range of concentrations in the "spiked" cultures, a relationship between the concentration of the test compound and its effect(s) may be obtained.

Preparation of Defined Medium--

A defined medium was prepared as described below. The compositions of the various stock solutions used are given in Table 7.

(a) 7.2 ml of S-1 and 36.0 ml of S-2 were added to a 4000 ml Erlenmeyer flask containing deionized water. The liquid level in the flask was increased to about 3000 ml with additional deionized water. The solution was then boiled over a Bunsen burner, while continually flushing with nitrogen gas at a flow rate of 1 liter per minute.

(b) The solution was cooled to room temperature, while continuing the nitrogen gas flushing. The following were then added to the solution: 24.0 ml of S-3, 2.4 ml of S-4, and 2.4 ml of S-5. An immediate black precipitate (FeS) was observed. The liquid level, which had been greatly reduced during the previous step, was then increased to 2400 ml with deionized water which had been boiled and cooled to room temperature under a nitrogen atmosphere.

(c) The flushing with nitrogen gas continued while flushing with a mixture of 30 percent carbon dioxide and 70 percent nitrogen (Matheson, Inc.) was begun. The CO_2/N_2 mixture was passed over hot copper turnings to remove any traces of oxygen in the gas. Once the new gas was flowing, the nitrogen gas flushing was ceased.

(d) 12.905 g of $NaHCO_3$ powder was added to the solution, resulting in 5.335 g/l of $NaHCO_3$. The CO_2/N_2 gas mixture was then bubbled through the solution, using a porous glass diffuser, until the pH stabilized at 7.1. The flask was stoppered and sealed with Parafilm, taking care not to contaminate the contents with oxygen. The solution was stored at room temperature until needed.

Table 7 Stock Solutions for Media Preparation in the Anaerobic Toxicity Assay

Solution	Constituent	Amount
S-1	$(NH_4)HPO_4$	26.70 g/l

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Table 7 (continued)

Solution	Constituent	Amount
S-2	$CaCl_2 \cdot 2H_2O$	16.70 g/1
	NH_4Cl	26.60 g/1
	$MgCl_2 \cdot 6H_2O$	119.98 g/1
	KCl	86.69 g/1
	$MnCl_2 \cdot 4H_2O$	1.35 g/1
	$CoCl_2 \cdot 6H_2O$	2.00 g/1
	H_3BO_3	0.38 g/1
	$CuCl_2 \cdot 2H_2O$	0.18 g/1
	$Na_2MoO_4 \cdot 2H_2O$	0.18 g/1
	$ZnCl_2$	0.15 g/1
S-3	Biotin	2.25 mg/1
	Folic acid	2.10 mg/1
	Pyridoxin·HCl	10.35 mg/1
	Riboflavin	5.15 mg/1
	Thiamine·HCl	6.30 mg/1
	Niacin	5.00 mg/1
	Pantothenate, Ca	5.80 mg/1
	Vitamin B^{12}	0.30 mg/1
	p-Aminobenzoic acid	4.95 mg/1
	Thioctic acid	5.15 mg/1
S-4	$FeCl_2 \cdot 4H_2O$	370.25 g/1
S-5	Na_2S	500.00 g/1

*All chemicals were of reagent grade purity; all solutions were made with deionized water.

The vitamins and minerals in this medium have been shown to be adequate for supporting methanogenesis (12). The initial assay concentrations of the major inorganic nutrients were: Nitrogen = 122 mg/l as N; Phosphorus = 19 mg/l as P; and Alkalinity = 2500 mg/l as $CaCO_3$.

Preparation of Substrate and Test Compound Solutions--

The final assay volume was to be 100 ml; thus, concentrated solutions of the methanogenic substrates and test compounds were needed if dilution of the inoculated medium was to be minimized. These solutions were prepared so that no more than 10 ml of the final volume (10 percent) would be contributed by them. In some cases, less than 10 ml was required, in which case deionized water was used to make up the difference.

The substrate solution was prepared such that 5 ml, when added to the culture sample, would result in an initial assay concentration of 1500 mg/l of acetic acid and 530 mg/l of propionic acid. In order to minimize losses due to volatilization and prevent a pH shock to the assay cultures, the substrate solution was neutralized to pH 7.0 with 1.0 N sodium hydroxide. 5.72 ml of glacial acetic acid (Eastman Kodak Co.) and 2.14 ml of propionic acid (J. T. Baker Co.) were added to deionized water in a 200 ml volumetric flask and neutralized. The liquid level was than brought to the mark with additional deionized water. The final concentrations of the stock solution were 30.00 g/l acetic acid (41.00 g/l sodium acetate) and 10.61 g/l propionic acid (13.76 g/l sodium propionate).

Solutions of the test compound were made from the stock solution described earlier. The final concentrations used to spike the assay cultures were: 120.0 mg/l PCP and 4.0 mg/l PCP.

Procedure for Starting the Assay

The inoculum for the methanogenic Toxicity assay was actively digesting sewage sludge from a nonacclimated laboratory reactor which had been fed only raw sewage sludge. The reactor was operated at an HRT (SRT) of roughly 30 days, and the digesting sludge had a suspended solids concentration of approximately 14,000 mg/l--half of which was volatile.

The flask containing the defined medium was placed in a 36°C constant temperature room (where the assay was to be run) to achieve thermal equilibrium. The flask was then opened and immediate gassing with the CO_2/N_2 gas mixture was begun (as before, an oxygen scrubbing unit was used to purify the gas). A graduated cylinder was used to measure out 600 ml of the inoculum sludge which was quickly added to the flask containing the medium. This resulted in a 20 percent inoculum by volume. A teflon-coated magnet was added to the flask and the flask was then placed on a magnetic stirrer to provide complete mixing of the medium. An apparatus was devised, using a 50 ml glass repeating pipet (L/I REPIPET, Labindustries, Inc.), with which it was possible to precisely pipet the inoculated medium while maintaining anaerobic conditions within the flask.

The assay was performed in 125 ml serum bottles (Wheaton No. 223748) which could be sealed with butyl rubber septa and aluminum crimp-type seals (Wheaton Nos. 224154 & 224183). A gassing cannula was flushed for about 30 seconds, at 2 l/min before adding the inoculated medium. The cannula was kept in the bottle during the addition of 90 ml of the medium, after which the cannula was removed as the butyl stopper/septum was put in place and the bottle sealed.

When all of the bottles contained inoculated medium, the appropriate amount of test compound was added using a 5 ml ground glass syringe. The solution was measured with the syringe and was injected into the bottle through the septum using a 20 gauge needle. If less than 5 ml of solution was required, deionized water was added to make up the difference, using the same procedure.

The final steps before beginning the assay involved the addition of the methanogenic substrates and the zeroing of the gas volumes in the bottles. A repeating syringe (Becton Dickinson and Co.) was used for precisely adding 5 ml of the substrate solution to each of the assay bottles. In the case of seed blanks, 5 ml of deionized water was added in lieu of the substrate, as previously described. The bottles were then equilibrated to atmospheric pressure, using the syringe technique described below, and placed on a shaker table in the constant temperature room. The initial concentrations of the test compounds and the volumes added to each bottle are summarized in Table 8.

Measurement of Gas Production Rates--

A syringe technique described by Owen et al. (12) was used to determine the gas production in each of the assay bottles. Twice each day, a water-lubricated, ground glass syringe was used to measure the volume of gas produced by equilibrating the gas in the assay bottle with atmospheric pressure. Syringes ranging in size from 1 ml to 20 ml were used, depending upon the volume of gas to be measured. Each was fitted with a 20 gauge needle. The syringe was held in a horizontal position while the needle was inserted through the septum. By gently twirling the water lubricated plunger, the gas pressure inside the assay bottle easily pushed the plunger outward until the volume change resulted in an equilization of pressures. The volume of gas produced was read directly from the calibrations on the syringe. The readings were double-checked by pulling the plunger past the equilibrium point and allowing it to return on its own, while gently twirling the plunger to reduce frictional resistance. Once the reading was made, the syringe was removed and the gas was wasted, thus zeroing the assay bottle to atmospheric pressure. The bottles were also zeroed at the start of the assay in the same manner. This method of gas measurement is very precise (about ± 1%) and with the proper choice of syringe size is also accurate. (The gas volume should be greater than 10 percent and less than 80 percent of the syringe capacity). The gas volumes were corrected to standard conditions by using the equation for correcting the daily gas production of the anaerobic reactors (Eq. (13)).

The gas was measured twice daily for about a week, after which time it was observed to level off dramatically. Presumably, this was due to a reduction of the methanogenic substrates to subsaturating concentrations. The analysis of the data collected in this assay is discussed in Chapter 6.

Analytical Techniques

Pentachlorophenol Analysis--

The procedure used to measure the concentration of PCP was described earlier in this chapter.

This extraction and derivatization technique gave reasonable recoveries of the PCP derivative from centrifuged anaerobic control reactor effluent.

Table 8 Initial Conditions for Methanogenic Toxicity Assay

Bottle	Identity	ml of PCP Stock		ml H_2O	ml Substrate
1	Control	0.0	-----	5.0	5.0
2	Control	0.0	-----	5.0	5.0
3	Control	0.0	-----	5.0	5.0
4	Control	0.0	-----	5.0	5.0
5	Seed Bank	0.0	-----	10.0	0.0
6	Seed Bank	0.0	-----	10.0	0.0
7	20 μg/l PCP	0.5	4.0 mg/l	4.5	5.0
8	20 μg/l PCP	0.5	4.0 mg/l	4.5	5.0
9	60 μg/l PCP	1.5	4.0 mg/l	3.5	5.0
10	60 μg/l PCP	1.5	4.0 mg/l	3.5	5.0
11	200 μg/l PCP	5.0	4.0 mg/l	0.0	5.0
12	200 μg/l PCP	5.0	4.0 mg/l	0.0	5.0
13	600 μg/l PCP	0.5	120 mg/l	4.5	5.0
14	600 μg/l PCP	0.5	120 mg/l	4.5	5.0
15	1800 μg/l PCP	1.5	120 mg/l	3.5	5.0
16	1800 μg/l PCP	1.5	120 mg/l	3.5	5.0
17	6000 μg/l PCP	5.0	120 mg/l	0.0	5.0
18	6000 μg/l PCP	5.0	120 mg/l	0.0	5.0

For quantification, an extracted standard with a concentration between 25 and 250 μg/l was used. This standard was made by adding a known amount of PCP (sodium salt) to centrifuged control effluent (thereby increasing the concentration of PCP by a known amount) and following the extraction/derivatization procedure outlined previously. A blank was also extracted and derivatized, starting with control effluent, to correct for PCP background in the reagents and control effluent. The calculation was performed as shown in Table 9.

The analysis for PCP was very reproducible down to about 10 μg/l, at which point integration of the peak areas became nonreproducible. The lower limit of instrument response using the electron capture detector was around 1 μg/l, but accurate and precise quantification was not possible below 10 μg/l. Interferences were occasionally a problem. It was found that baking all glassware prior to use or preextracting with benzene eliminated most of the trouble. (Separatory funnels washed with Alconox were later found to be a common source of contamination, but baking at 350°C drove off the problem residues). Use of only freshly made reagents was critical, as the chloroacetic anhydride, in particular, can degrade with storage. The benzene was redistilled, which eliminated it as a source of contamination and conserved benzene. It was found that benzene which, upon injection, resulted in a "peak-free" chromatogram was suitable for use in the analysis.

Total Alkalinity (TA)--

Table 9

Quantification of Extraction/Derivatization Procedure for Pentachlorophenol

Applied PCP Concentration in sample (μg/1)	Percent recovery using an absolute standard of PCP-chloroacetate in benzene for calculation		Concentration in sample using extracted/derivatized sample for standard (initial PCP=200 μg/1)	Average percent recovery using extracted/derivatized sample for standard
	X	σ		
250	64.9	5.0	259.4	103.7
125	63.8	2.9	127.4	101.9
25	61.6	5.6	24.6	98.6

Calculation of the concentration of PCP in an unknown sample was performed as follows.

$$\mu g/1 \text{ PCP in sample} = \frac{\text{Standard concentration } (\mu g/1)}{(PA_{std}) - (PA_{control})} \text{ X } PA_{sample} \qquad (16)$$

where,

The standard is made by spiking the control sample with PCP prior to extraction/derivatization.

PA = integrated peak area (average).

The total alkalinity of the digesting sludge was determined by the titration procedure in Standard Methods for the Examination of Water and Wastewater. (11) The samples were centrifuged for 15 minutes at 18,400xG and were transferred to 25 ml glass vials for storage at 4°C. A sample of 10 ml was titrated to pH = 4.0 using a standardized 0.1xxx N H_2SO_4 solution. The pH was measured with a Fisher Accumet model 600 pH meter. Storage of samples for only a few days in sealed vials resulted in no changes in the measured alkalinity; however, storage of samples for longer periods resulted in more significant changes (greater than 10 percent). Therefore, samples were generally analyzed within two weeks of sampling.

Total Volatile Acids (TVA)--

The procedure used for determining the concentration of volatile acids was the same as in Standard Methods for the Examination of Water and Wastewater. (11) The method used was a chromatographic separation of volatile acids on silicic acid, followed by elution with a chloroform/butanol solvent and titration to the phenolphthalien endpoint with methanolic sodium hydroxide. Although the procedure was the same as in Standard Methods, it was found that the vacuum source for eluting the acids from the column greatly affected the results. A vacuum pump, for example, gave consistently lower results--presumably due to stripping of the acids under the low pressure. When a water aspirator was used, values were increased, but remained quite variable. Carefully controlling the vacuum to about the same value helped the precision of the analysis. Measurement of standard solutions of sodium acetate gave excellent precision and good accuracy when all variables were closely controlled, and was performed as a routine check of the accuracy of the procedure. Samples stored in sealed vials (where pH = 7.5-8.0) for as long as two months showed no difference upon analysis from the same samples analyzed immediately after centrifugation. Toward the end of this research it was necessary to reuse silicic acid. By drying overnight in a hood followed by baking in the muffle furnace at 550°C for 2 hours, the silicic acid powder was cleaned and reactivated. Using recycled silicic acid had no apparent effect upon the analysis.

Gas Analysis--

The off gas was analyzed to determine the percentages of methane, carbon dioxide, and nitrogen. No attempt was made to measure hydrogen, hydrogen sulfide, or other products of the anaerobic process. The instrument used in this analysis was a Fisher model 25V gas partitioner driving a Houston Instruments Omniscribe model B-5000 strip chart recorder. The gas partitioner contained two columns for the separation of gases The first column was developed by Supelco, Inc., for separations based upon polarity of the gases (30% SP-1400 on 80/100 Chromosorb P-AW; 6 feet, 1/4", aluminum). The second column separated gases by molecular weight and adsorbed CO_2 irreversibly under the operating conditions. (Molecular Sieve 13X, 45/60 mesh; 6 feet, 3/8", aluminum). The operating conditions of the instruments were:

Temperature	Ambient (22-25°C)
Carrier gas flow rate (He)	80 ml/min (20 psig)
Detector cell current	HIGH (7 mAmp)

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Sensitivity	2 percent
Recorder sensitivity	1 mV full scale
Sample size	0.5 ml at ambient pressure

A 5 ml glass syringe, with a teflon-seal plunger, was used for gas sampling. A 22 gauge needle was placed on the syringe and inserted through the septum in the gas line (Figure 6) to sample the reactor gas space. The syringe was flushed several times before taking a 5 ml sample of gas. The syringe was then carried to the partitioner. During the time between sampling and injection, a small amount of pressure was kept on the syringe plunger to ensure no inward flow of gases The plunger was then pushed to the 0.5 ml mark, held for 5 seconds to allow pressure equilization, and the sample was injected. Reproducibility within 5 percent was possible with this technique.

Quantification was done by comparing peak heights of the individual constituents in the sample with peak heights of the same constituents in a standard gas mixture of CO_2 and CH_4. (Air was used for nitrogen and oxygen determinations, assuming 78% N_2 and 20.5% O_2 in wet air). The standard gas mixture was 29.975% CO_2 and 70.025% CH_4, and was obtained from Matheson, Inc. Individual constituents constituting less than about 5 percent of the sample gas could not be determined except by difference. The calculation was performed as follows.

$$\text{Percent GAS in sample} = \frac{\text{Peak Height GAS in Sample}}{\text{Peak Height GAS in std.}} \times \begin{array}{c}\text{percent GAS}\\ \text{in std.}\end{array} \qquad (17)$$

where,

GAS = any individual constituent, e.g. CO_2, CH_4, N_2, etc.

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CHAPTER 6

EXPERIMENTAL RESULTS AND DISCUSSION

In the Introduction to this report three questions were posed which had to be answered before a long-term research commitment on the testing of priority pollutants could be undertaken. Those questions were addressed in the preceding chapters, culminating in the protocols proposed for aerobic and anaerobic testing. This chapter evaluates the efficacy of those protocols; a task, which in the final analysis, required an evaluation of whether they did indeed provide information of the sort needed. As stated earlier, PCP was chosen as the priority pollutant with which that evaluation was made. This choice was fortuitous because the unique characteristics of the compound required some reconsideration of the proposed methodology at an early stage in the work. Although this proved to be time-consuming it was rewarding because of the confidence it engendered in the ability of the protocols to meet the stated objectives.

AEROBIC TESTING

In order to meet the objectives of this research seven specific questions were posed and aerobic experiments were designed to answer them:

1) Using the culture system described as the Phase I acclimation reactor can a biomass be acclimated to provide a significant reduction in the wastewater PCP concentration?

2) Based on operational experiences, what modifications in system design are needed for Phase II CSTR's?

3) What is the effect of SRT on "steady-state" levels of PCP in Phase II CSTR's? (The reader should note that it was observed that concentrations of organics and concentrations of PCP were shown to effect the "steady-state" levels of PCP; therefore, these effects had to be evaluated).

4) How can the data generated be used to predict PCP degradation in full-scale systems?

5) What effect does PCP have on "normal" performance of the Phase II system?

6) Is it feasible to use the degradation of an internal standard to estimate PCP degradation in a full-scale system?

7) How do competing biological-physical-chemical removal systems affect the disappearance of PCP in Phase II systems.

Phase I Testing

Fiber wall reactors as shown in Figure 3 were operated for 2 1/2 months with an input flow containing domestic wastewater supplemented with dog food extract to give an SCOD of 200 mg/l. PCP as the sodium salt was added in specified concentrations which were increased periodically from 1 mg/l to 20 mg/l. The wastewater was also supplemented with LAS, if needed, to give a concentration of 10 mg/l as measured by the methylene blue active substances (MBAS) test. Effluent PCP concentration was measured quite often in the final month of acclimation and was 100 µg/l or less. Since air stripping of sodium PCP was found to be negligible and adsorption isotherms on glass and on unacclimated biomass could not account for the reduction in PCP concentration, this pollutant was elevated to second level testing. Data for adsorption and air stripping will be presented later. It should be noted that spot checks of SCOD concentration and MBAS concentration resulted in values of around 40 mg/l for the former and 0.4 mg/l for the latter.

The relative ease of acclimation in Phase I indicates that organisms existed in the seed stock with the genetic capability for degrading PCP and that the acclimation procedure was satisfactory for hastening the growth of those organisms. Several published reports seem contradictory to this. For example, Heidman, et al., (13) were unable to acclimate a biomass when PCP at 10 mg/l was used initially in the influent with incremental daily increases of 10 mg/l PCP. Watanabe (14) showed that PCP at a concentration of 10 mg/l significantly inhibited the growth of PCP-degrading organisms. We have observed in our own labs that viable plate counts of a PCP degrading culture, Arthrobacter KC3, are decreased 38% at 12.5 mg/l and 55% at 25 mg/l PCP. Consequently, it appears critical to start acclimation at low concentrations of priority pollutant and to increase the concentration very gradually to the required input level. Ideally, increases in the influent concentration should be attempted only after evidence is obtained that removal is occurring at the existing influent concentration.

The proposed protocol requires that the priority pollutant be subjected to extensive Phase II testing if alternative removal mechanisms such as adsorption and volatilization cannot account for the observed removal of the compound in Phase I. Consequently, consideration was given to the possibility that these nonbiological removal mechanisms might be responsible for the observed PCP removal.

One should recognize the difficulty of distinguishing the removal of priority pollutant by adsorption to the biomass from removal by metabolic attack, when organisms capable of PCP degradation may inhabit the biomass. Ideally, one might inactivate the biomass, expose it to PCP, separate and wash the solids, extract the PCP quantitatively and measure the extracted PCP. There are obvious problems with this approach since drastic measures may be required to inactivate the biomass and these may significantly alter its adsorptivity. Furthermore, the extraction methodology may not be quantitative or reproducible. Alternatively, one might use unacclimated biomass provided that it is reasonably representative of the acclimated biomass. Under the test conditions employed, in which the organic carbon contributed by the PCP constituted less than 10 percent of the organic carbon in the feed, one would expect the bulk of the biomass to have arisen from the background organic matter rather than from the PCP. This suggests that the use of unacclimated biomass may be a reasonable approach. To evaluate this, unacclimated biomass from the control reactor was compared with acclimated biomass from the reactor receiving PCP to determine the characteristics of PCP removal in each. This was done by adding PCP at 500 µg/l to a 1000 mg/l slurry of each biomass. Soluble PCP concentrations were determined over a period of 20 hours and the results are graphed in Figure 7. The biomass incapable of degrading PCP removed it rapidly from solution, reducing the concentration from 500 µg/l to approximately 200 µg/l within about one hour. After this initial removal the PCP concentration did not change for the remainder of the experimental period, suggesting that adsorption was the mechanism of removal. In the case of the PCP-acclimated cells, however, the PCPconcentration decreased rapidly and completely disappeared with 2-6 hours. This result was interpreted as a combination of adsorption and degradation.

Using unacclimated biomass, an experiment was done to obtain data which would allow prediction of the adsorptive capacity of biomass at equilibrium conditions. This involved adding various amounts of control reactor solids to flasks containing various concentrations of PCP in control reactor effluent at a pH of 8.1, identical to that in the reactors. The cell masses used were selected to give approximately 50% reduction in PCP concentration using an assumed adsorptive capacity estimated from the single flask test just described. The following combinations of cell and PCP concentrations were used:

Initial PCP Conc. (µg/l)	Cell Concentration (mg/l)
100	1000
500	1000
2000	2000
5000	3000
10000	3000

The slurries were mechanically mixed for 20 hours, after which the equilibrium PCP concentrations were measured by G-C analysis. The amount of PCP adsorbed onto the solids was determined by the difference between the initial and final concentrations. The specific capacity at the various equilibrium PCP concentrations was calculated by dividing the removal of PCP by the cell concentration used. Figure 8 shows the results of these determinations. The curve is a typical capacity-concentration relationship for adsorption onto solid surfaces with increased capacity at higher equilibrium concentrations of solute. The reduction in slope is suggestive of the tendency to saturate binding sites for PCP. Attempts to fit the data to the Freundlich and Langmuir adsorption models were unsuccessful. However, the experimental curve allowed prediction of the extent of adsorption of PCP onto biomass for various equilibrium PCP concentrations and it was used in both Phase I and II to estimate the importance of the adsorptive mechanism for PCP removal

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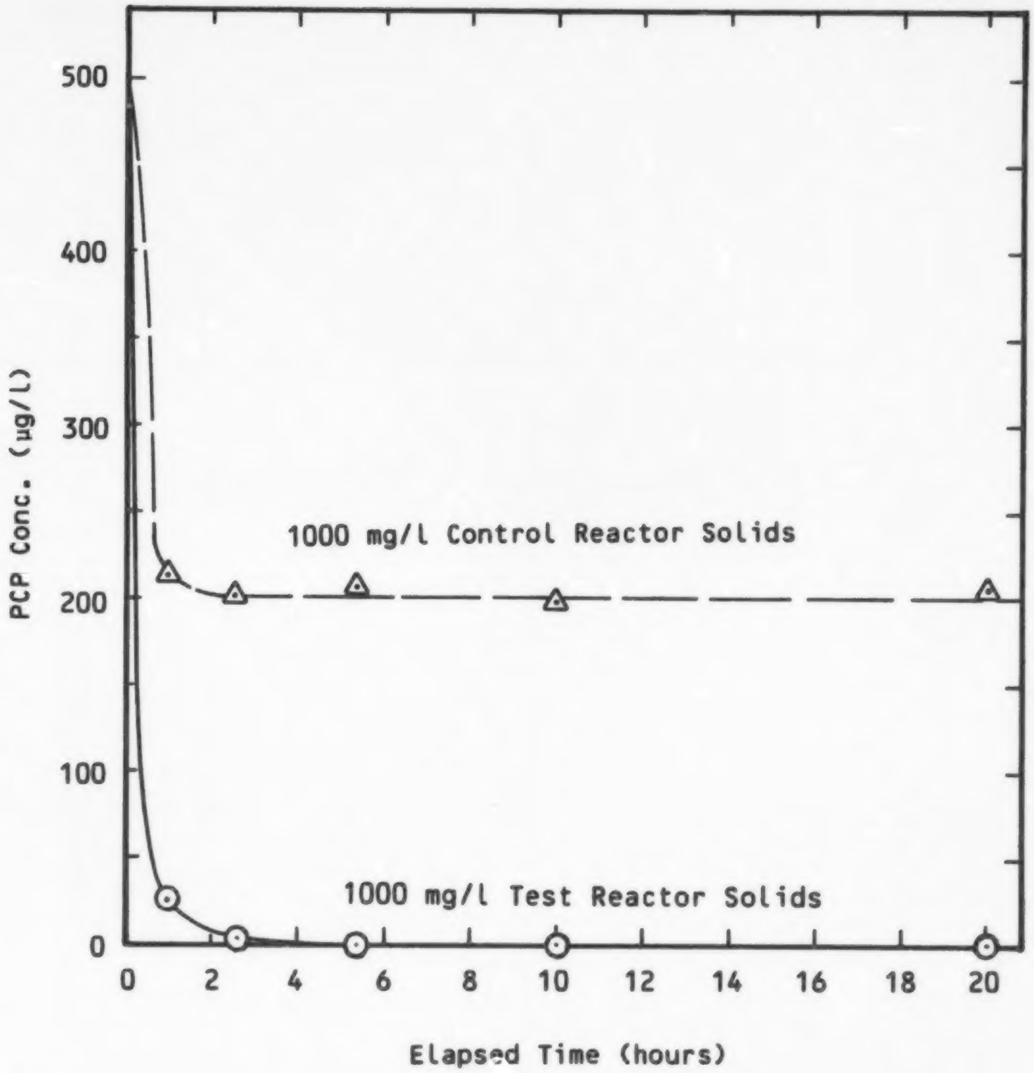

Figure 7 PCP removal by acclimated and unacclimated biomass.

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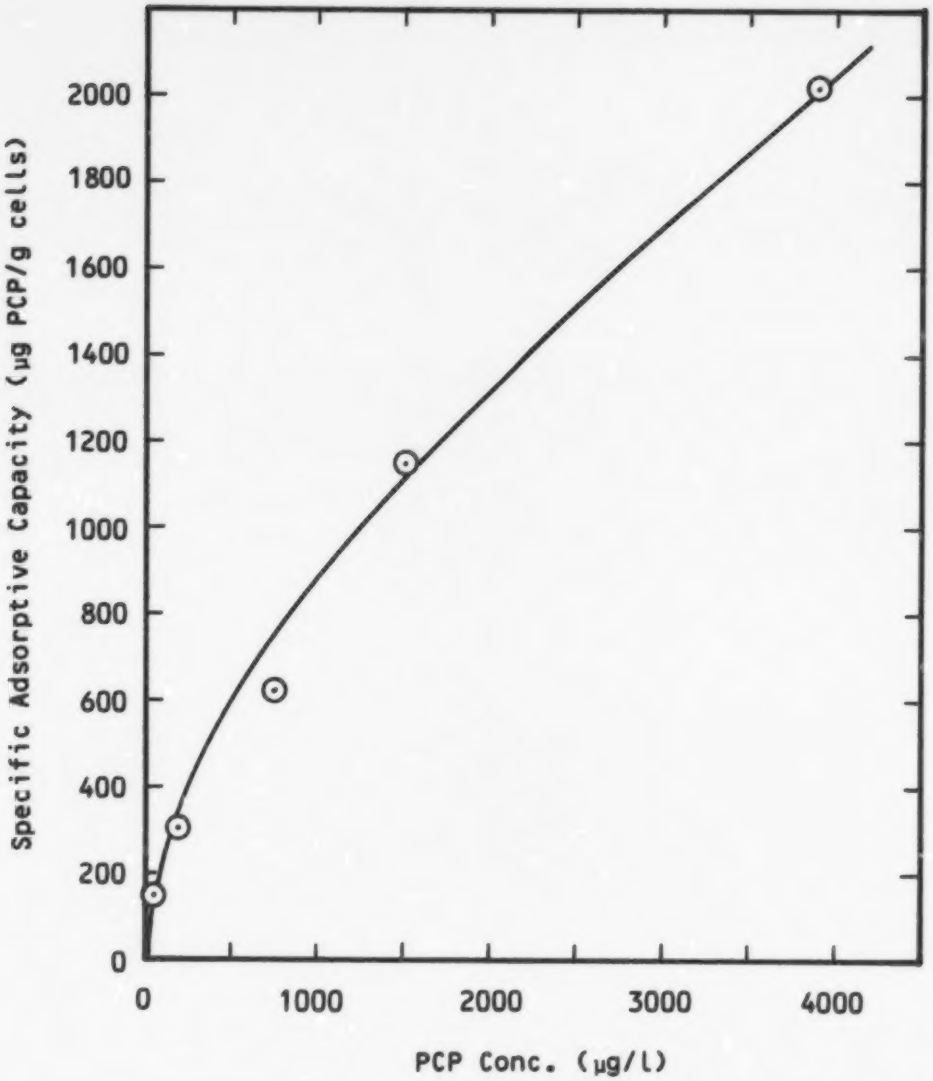

Figure 8 Isotherm for adsorption of PCP onto unacclimated biomass.

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relative to the metabolic mechanism.

Since no biomass was removed from the fiber wall reactor during the acclimation period, a situation would develop in which the biomass would be in equilibrium with the soluble PCP concentration with respect to adsorption. Under that condition, as long as the soluble PCP concentration remained constant, the only biomass available for adsorption would be that formed each day. Thus the contribution of adsorption to PCP removal may be estimated by applying the specific adsorptive capacity associated with the reactor soluble PCP concentration to the mass of new cells formed daily. The PCP concentration in the reactor was less than 100 μg/l. Thus, from Figure 8, a conservative value for the specific adsorptive capacity is 200 μg/g. The SCOD of the feed was approximately 200 mg/l and the feed flow rate was approximately 8 liters/day. The true growth yield on the organic matter was 0.45 (to be discussed later) so that the maximum amount of biomass formed daily would be in the neighborhood of 0.72 grams. The actual amount would be less than that due to the importance of cell decay at the long SRT employed, but the use of the 0.72 g/day figure will cause any error to be in favor of adsorption. Multiplying the 0.72 g/day of new biomass by a specific adsorptive capacity of 200 μg/g gives an adsorptive loss of PCP of 144 μg/day. The PCP concentration in the feed to the reactor was 20 mg/l after acclimation was achieved, and thus the mass input was 160 mg/day. Thus adsorption contributed less than 0.1% to PCP removal.

A long term air stripping test was performed to determine the extent of volatilization of PCP from the reactor contents. This was done by spiking filtered and autoclaved control reactor effluent (from Phase II testing), pH 8.0, with 100 μg/l PCP in a sterile reaction vessel. The vessel was aerated by passing a stream of sterile, humidified air through fritted glass diffusors in the bottom of the reaction vessel. Any water lost by evaporation was replaced daily with sterile deionized water. Samples for PCP analysis were removed from the vessel through a rubber septum using a large stainless steel hypodermic needle and plastic syringe. The experiment was allowed to continue for two weeks, and samples were taken for PCP analysis every 2 or 3 days. Figure 9 shows the loss of PCP during this period. As shown, the PCP concentration decreased only slightly. Using this data, a first-order rate constant for the stripping process was determined by plotting the natural log of the PCP concentration versus time and measuring the slope. The value was 0.0076 day^{-1}. Assuming that the mass transfer characteristics of the stripping test reactor are similar to those of the acclimation reactor, the rate constant can be used to estimate the contribution of stripping to the removal of PCP. Multiplying the rate constant by the reactor PCP concentration of 100 μg/l gives a volumetric stripping rate of 0.76 μg/l·day. Since the liquid volume inside the fiber-wall container was approximately 4 liters, this corresponds to a total rate of 3.0 μg/day. This is negligible in comparison to the input rate of 160 mg/day. In fact, even if the rate constant were underestimated by two orders of magnitude the rate would still be negligible. Thus it was not considered necessary to perform a more refined measurement of the mass transfer coefficient.

Having determined that significant amounts of PCP were being removed in

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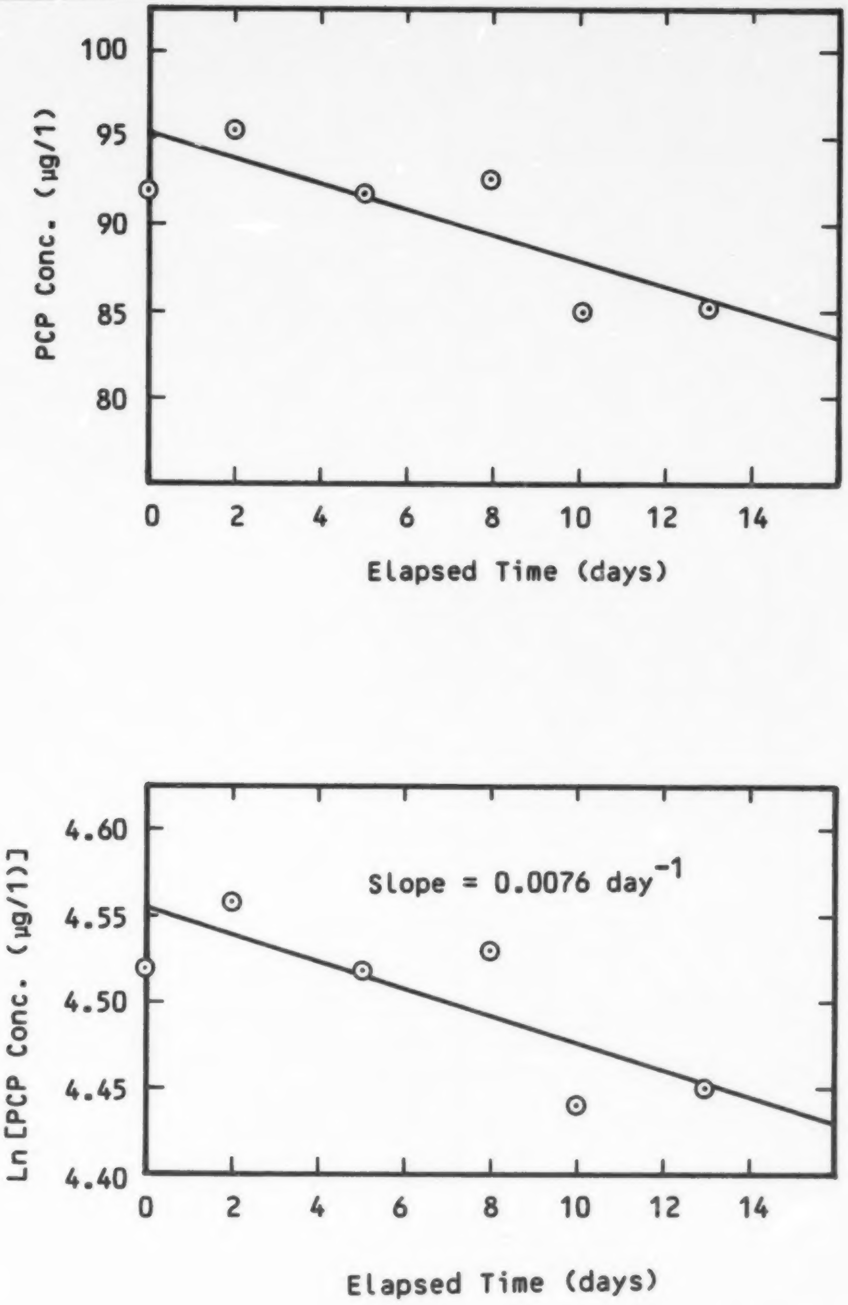

Figure 9 Loss of PCP from sterile reaction vessel by stripping.

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the acclimation reactor and having shown that neither adsorption nor stripping could account for the loss, it must be concluded that an acclimated biomass had been developed and that at least primary biodegradation of PCP was occurring. Consequently the decision could be made to proceed to Phase II testing.

Phase II Testing

As indicated in the logic diagram (Figure 1) during Phase II testing four reactors were operated on a PCP wastewater at solids retention times (SRT) of 3, 7, 11, and 15 days. In addition, four control reactors were fed wastewater without PCP while operating at the same SRT's. The testing period for PCP was unusually long because a number of equipment adjustments were made to smooth out operational difficulties. These included: 1) redesigning the effluent overflow tubes to prevent irregular loss of solids through foam fractionation, 2) increasing the feed COD to increase the cell concentration in the reactors, 3) eliminating the effects of photosynthetic activity in the reactors, 4) shortening the influent lines and converting them primarily to glass, and 5) correcting for evaporative losses.

Alterations to Original Reactor Design--

In the first few weeks of operation, several problems developed which had to be corrected. The suspended solids concentration declined sharply in some of the reactors, particularly those with a 7 day SRT. An analysis of the reactor effluent revealed that the solids concentration in it was higher than in the reactor. This may have been primarily due to the aeration device and the type of effluent overflow tube because the small air bubbles tended to concentrate the cells at the liquid surface where they were carried out the overflow tube. This problem was solved by installing a small (3 mm dia.) glass siphon tube at the top of each outlet tube. The siphon caused the liquid depth to increase until the head in the siphon was overcome, a depth of approximately 1/4 inch. The siphon would then discharge until air was drawn in. This allowed the effluent to be removed two to five times per day in discrete, high flow rate discharges. The higher liquid flow rate and the removal of effluent from below the surface, where the turbulence was greater, acted to make the effluent solids concentration the same as that in the reactor. The siphon also eliminated the selective removal of surfactants that occurred because of foam formation. The main problem with the siphon system was that it caused a slight variation in the mass of cells in the reactor, because of the volume build up between discharges. The discharge volumes were only 2-5% of the total reactor volume, however, and long term testing tended to diminish any effect this might have had.

Another problem was that cell material tended to either settle out or become attached to the bottom and sides of the reactor. In one instance, 40% of the total cell mass became immobilized on the reactor walls. Since the immobilized cells were not carried out in the effluent, this resulted in a buildup of cell mass in the reactor. The higher cell mass increased the SRT and depressed the effluent SCOD, PCP and MBAS concentrations below what they would have been if the reactor contents had been completely suspended. The impact of solids immobilization was lessened by daily wall cleaning.

Furthermore, the influent SCOD concentration was increased to 600 mg/l thereby increasing the suspended cell concentration in the reactors and decreasing the fraction of cells which were immobilized on the walls between cleanings, thereby reducing its significance. The SCOD was stepped up in 100 mg/l increments and remained at 600 mg/l for the rest of the study.

The third problem encountered was the outgrowth of a strain of dark brown diatomaceous alga. These organisms were much more prevalent in the control reactors and at longer SRTs. Consequently, the reactor walls and lids were painted black, stopping further growth of the diatoms. The dead diatoms were carried out in the effluent, a process which took over a month because of the long SRTs used.

Another problem was caused by growth in the feed lines. When the SCOD of the feed actually entering the reactors was measured it was discovered that over 70% of the SCOD present in the feed in the refrigerated reservoirs has been removed by the time the feed reached the reactors. Furthermore this removal was greater where silicone rubber tubing was used rather than tygon. MBAS was reduced by a similar amount although PCP was not affected. These losses caused the concentration of feed components to vary and also caused large numbers of cells to be present in the influent, both of which disrupt steady-states and make kinetic analysis uncertain. Although growth in the feed lines could not be eliminated because of the sewage in the feed, steps were taken to minimize its effects. The pump was placed on a platform above and between the two sets of reactors it supplied, thus minimizing the tubing lengths from the pump to the reactors. The refrigerator holding the feed bottles was placed directly behind the pump, greatly reducing the length of tubing from the feed jugs to the pump. All of the flexible tubing from the feed bottle to the pump was replaced with small (2 mm ID) glass tubing. The tubing from the pump to the reactors was replaced with 6 mm I.D. glass tubing. This large diameter tubing allowed the feed to trickle down to the bottom, greatly reducing the liquid's residence time in the tubing. These changes reduced the loss of SCOD from more than 70% to less than 20%.

The loss of SCOD was carefully analyzed over a typical tubing cleaning cycle and the results are shown in Figure 10. The upper two curves show the SCOD concentration in the feed bottles while the lower two curves show the SCOD concentration of the feed as it entered the reactors. The reduction in the feed bottles was slight and occurred at a relatively constant rate. The SCOD entering the reactors underwent more drastic changes which depended upon the time in the cleaning cycle. At the start of a cycle, newly prepared feed was placed in the refrigerator and the glass tubing from the feed pump to the reactors was cleaned in soap and water. Feed pumped through this tubing exited at a SCOD of approximately 530 mg/l (compared to 600 mg/l in the feed jug). As wall growth built up in the tubing, the SCOD dropped to 420 mg/l and remained roughly constant. The day after the feed was changed, the tubing was again cleaned but the feed was not replaced. During this cleaning, the silicone rubber tubing used in the pump was replaced with clean tubing. After this cleaning, the pattern of SCOD change was similar to that following the first cleaning, in which the pump tubing was not replaced. A time-weighted average of this SCOD data revealed that the control reactors were receiving 430 mg/l of soluble COD and PCP test reactors were receiving

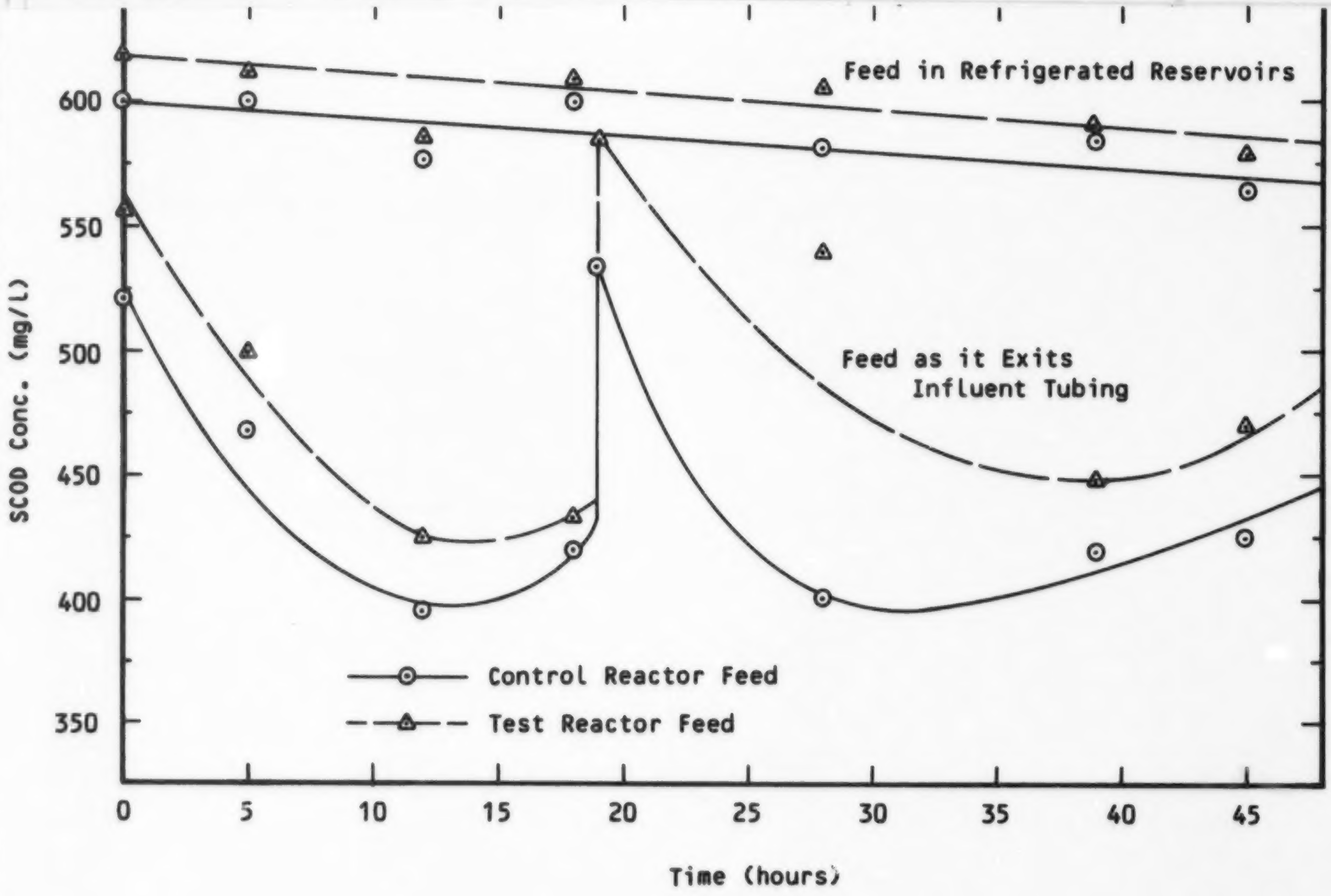

Figure 10 Change in feed SCOD during a typical feeding cycle.

470 mg/l on the average. Furthermore, the SCOD varied from a maximum of 530 mg/l to a minimum of 400 mg/l for the controls and from 580 to 420 mg/l for the test reactors. The difference was due to the presence of PCP in the test reactor feed.

Because the reactors were mixed by aeration, relatively high air flow rates were required. Thus, despite the fact that the compressed air supply was humidified before entering the reactors, a significant evaporation loss occurred. By collecting and measuring the effluent volumes, the magnitude of this loss was determined, thereby allowing correction of the SRT in the reactors. Table 10 shows these corrected values for both the test and control reactors. For the sake of consistency, we will continue to refer to the reactors by their nominal SRT values, however. This evaporation also tended to concentrate the insoluble or nondegradable soluble matter in the reactors. This effect has little significance to the normal operational parameters. It does effect some calculations involving suspended solids concentration, as will be discussed later.

Table 10 Measured Reactor Solids Retention Times

Reactor No.	Nominal SRT days	Effluent Volume ml/day	Reactor Volume liters	Measured SRT days
1	3	470	1.5	3.19
2	7	450	3.5	7.78
3	11	430	5.5	12.79
4	15	410	7.5	18.29

Continuous Flow Reactor Performance--

As a result of the modifications to the reactors, relatively stable operational periods ensued and final data collection was begun. Figures 11 to 18 present the data collected over the five month period following modification of the reactors.

Several general observations should be made. First, it is clear that except for some minor perturbations, particularly in the lowest SRT reactors, concentrations of SCOD, SS and MBAS stabilized quite nicely throughout the operating period. The COD removal efficiency usually exceeded 95% with the result that the effluent concentration was below 30 mg/l. Secondly, with the exception of reactor R4 (SRT - 15 days), the SCOD in the control reactors and in the PCP reactors showed a tendency to decrease with increasing SRT as Monod kinetics would predict. It was interesting to note that reactor R4 contained dense granular floc not seen in the other reactors. When examined under the microscope, the floc consisted of a central core of bacteria entirely surrounded by rotifers typical of the genus, Philodena. It was postulated that a mass transfer limitation into the dense central core might be the cause of the higher SCOD in the reactor. Finally, it should be noted that several spikes in effluent SCOD can be observed for both the PCP and the control reactors. These have been attributed to operational episodes

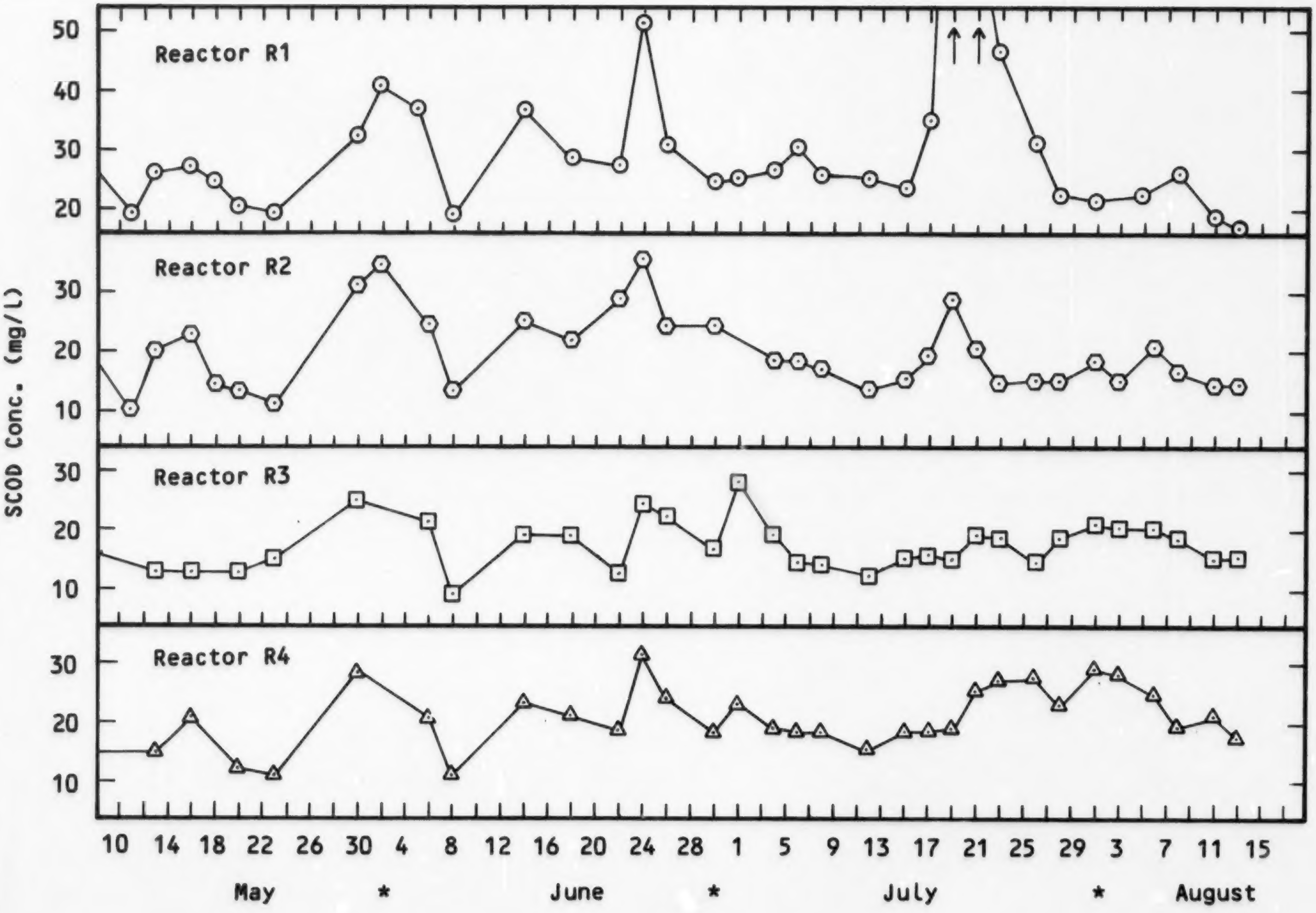

Figure 11 SCOD concentration in test reactors during Phase II testing.

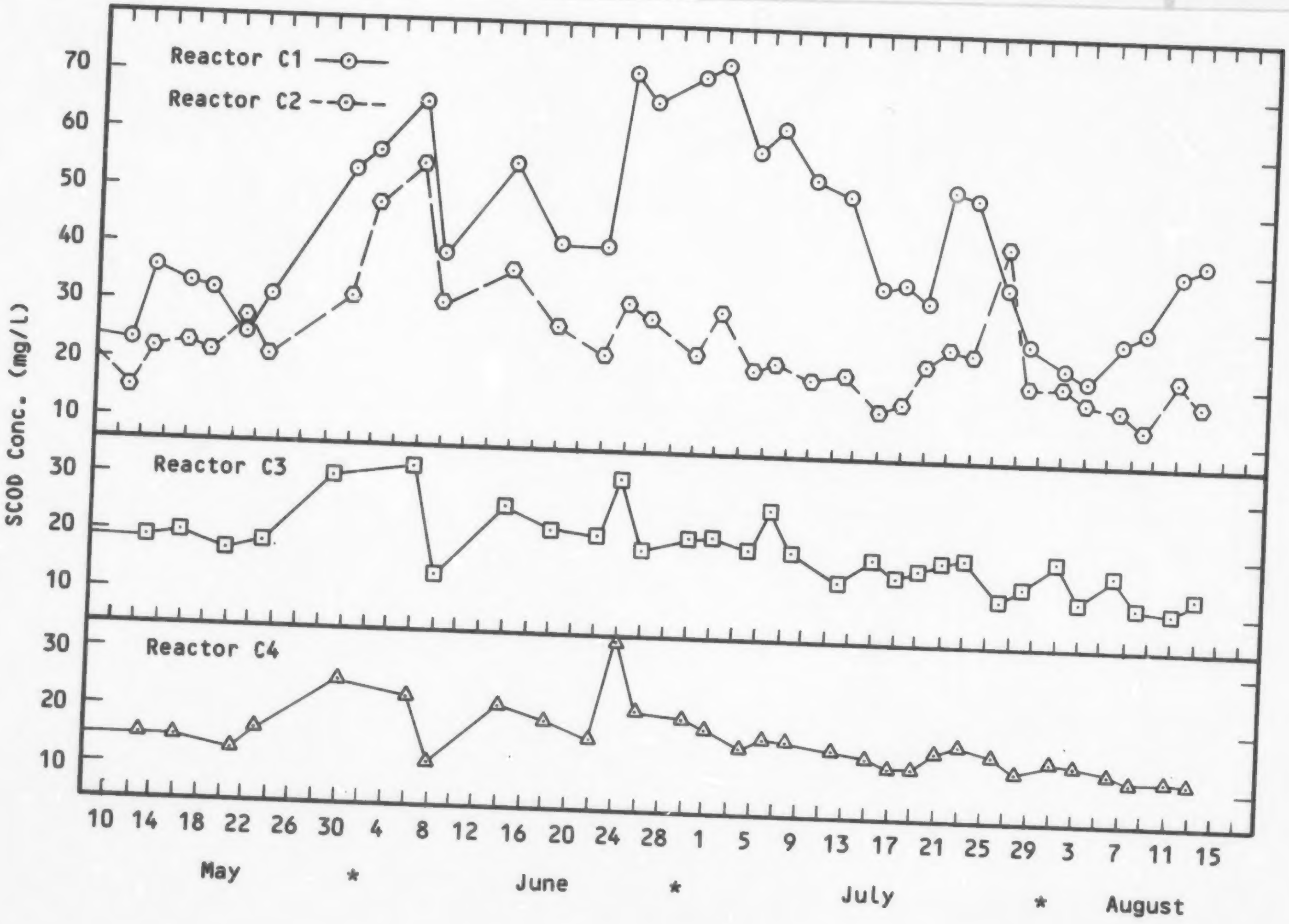

Figure 12 SCOD concentration in control reactors during Phase II testing.

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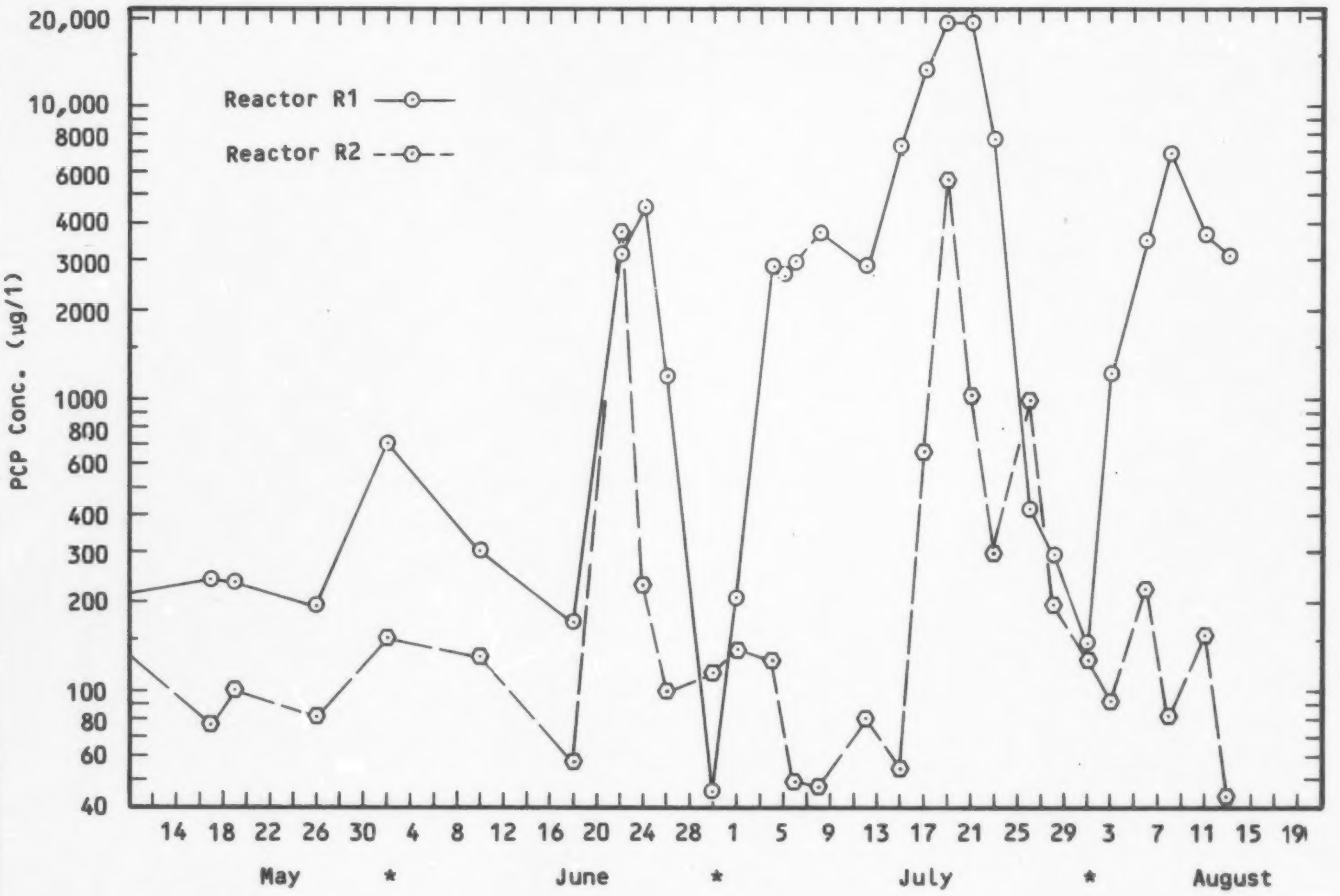

Figure 13 PCP concentration in test reactors 1 and 2 during Phase II testing.

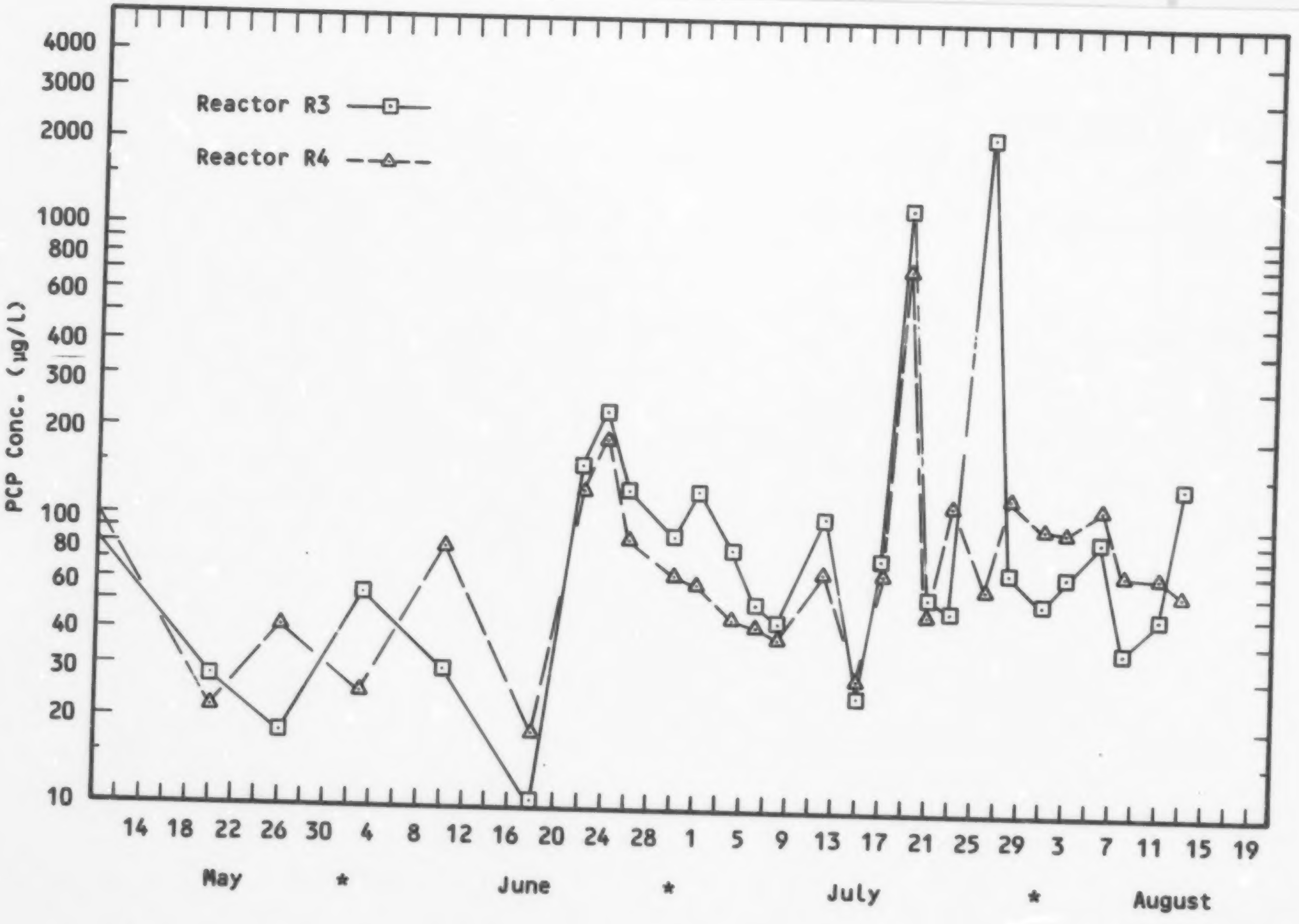

Figure 14 PCP concentration in reactors 3 and 4 during Phase II testing.

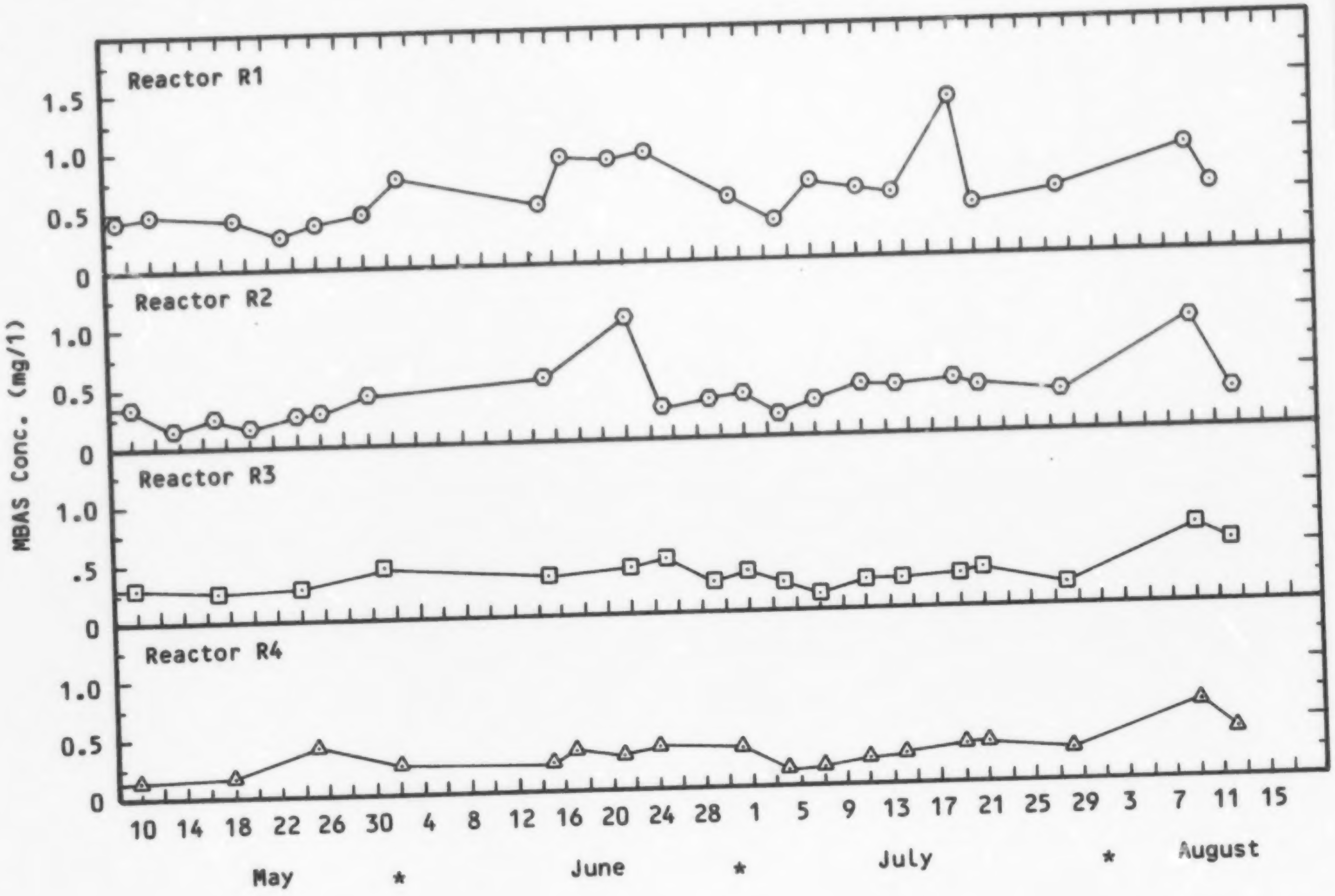

Figure 15 MBAS concentration in test reactors during Phase II testing.

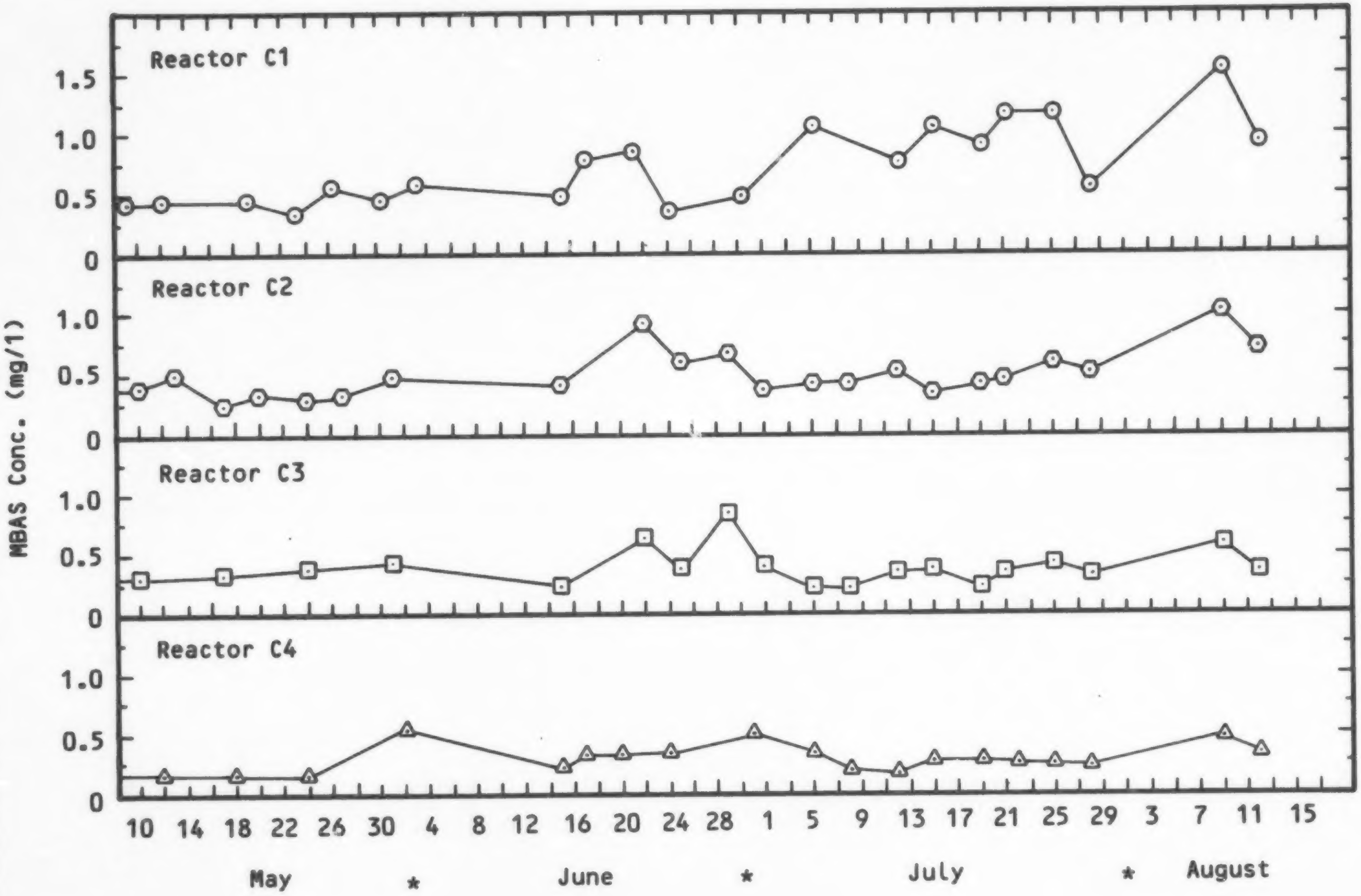

Figure 16 MBAS concentration in control reactors during Phase II testing.

which stressed the reactors for short periods of time.

Figures 13 and 14 describe the changes in PCP concentration with time. It should be noted that the PCP concentration is plotted on a log scale in order to accommodate the 500-fold range of concentrations encountered. A high degree of variation in effluent concentration was noted, although considerably greater stability was achieved at longer SRT's. Reactor R1 (SRT = 3 days) was most unstable with PCP varying between 45 µg/l and 20,000 µg/l. The periods of highest PCP concentration seemed to correlate with periods of highest SCOD concentration, although the magnitude of the increase was far greater for PCP than for SCOD. It is important to note, however, that given time, the systems invariably recovered with restoration of both SCOD and PCP degradation. Apparently the biological system was able to adapt to increasing PCP concentration. The irregularities in response in reactor R1 (and perhaps R2) make it difficult to argue that the reactors achieved steady state, and from a practical point of view, suggest that the SRT must exceed 7 days for consistent PCP degradation. In spite of these variation, the mean level of PCP in the reactor effluents decreased with increasing SRT, even though during some periods of operation the PCP concentration in reactor R4 was higher than in reactor R3. This was particularly true when the floc in reactor R4 was dense and heavily burdened with rotifers.

With respect to MBAS, effluent levels are shown in Figures 15 and 16. There was considerably more stability in MBAS than for the other parameters measured and once again, MBAS concentration decreased with increasing SRT's in accordance with Monod kinetics.

Figures 17 and 18 show the suspended solids data and suggest that after the first month of operation cell levels were fairly constant from day to day and between control and test reactors. Furthermore the tendency was for the suspended solids concentration to decrease with increasing SRT, which is typical of biological reactors operated at long SRT's due to the importance of cell decay.

Determination of Steady-State Values--

It is clear that the reactors approached steady state only toward the end of the operational period, and consequently, only data from the last few months of operation were used to determine steady-state values. Since the data represented a randomly distributed set of non-negative values grouped around a sample mean, log-normal distributions were expected. Therefore, the data were plotted on log-normal probability paper. In every case COD, SS and MBAS data followed a straight line indicating the validity of using a log-normal distribution. PCP data below 350 µg/l also fit a log-normal distribution, however, data above 350 µg/l were not log-normally distributed. The probability plots for PCP are shown in Figures 19-22. The reason for this will become apparent later. Only data with a PCP concentration of 350 µg/l or less were used to calculate the mean values. Extremely low data points which did not fall on the line were also excluded.

The selected data from each reactor were used to calculate the log-mean

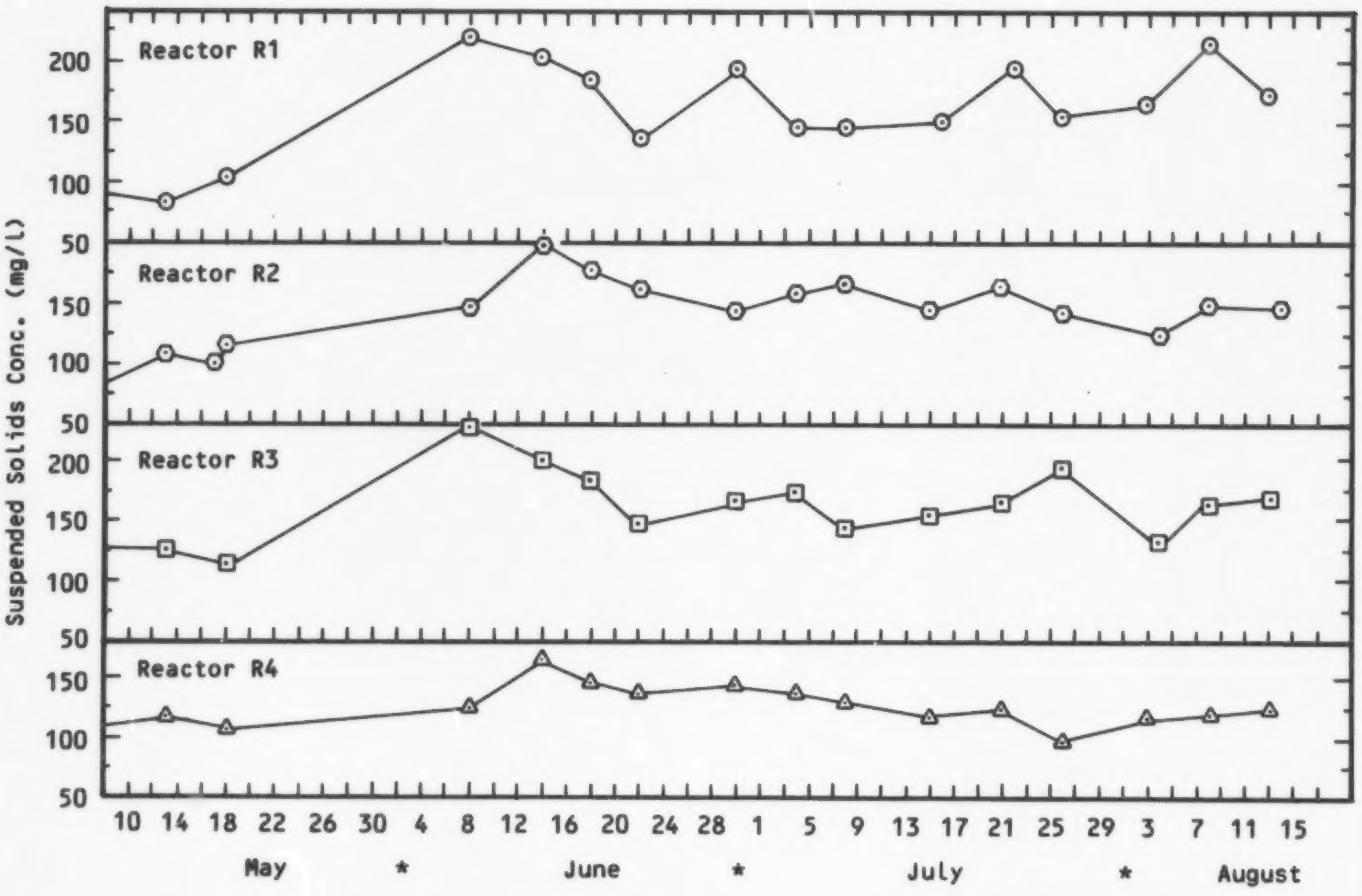

Figure 17 Suspended solids concentration in reactors during Phase II testing.

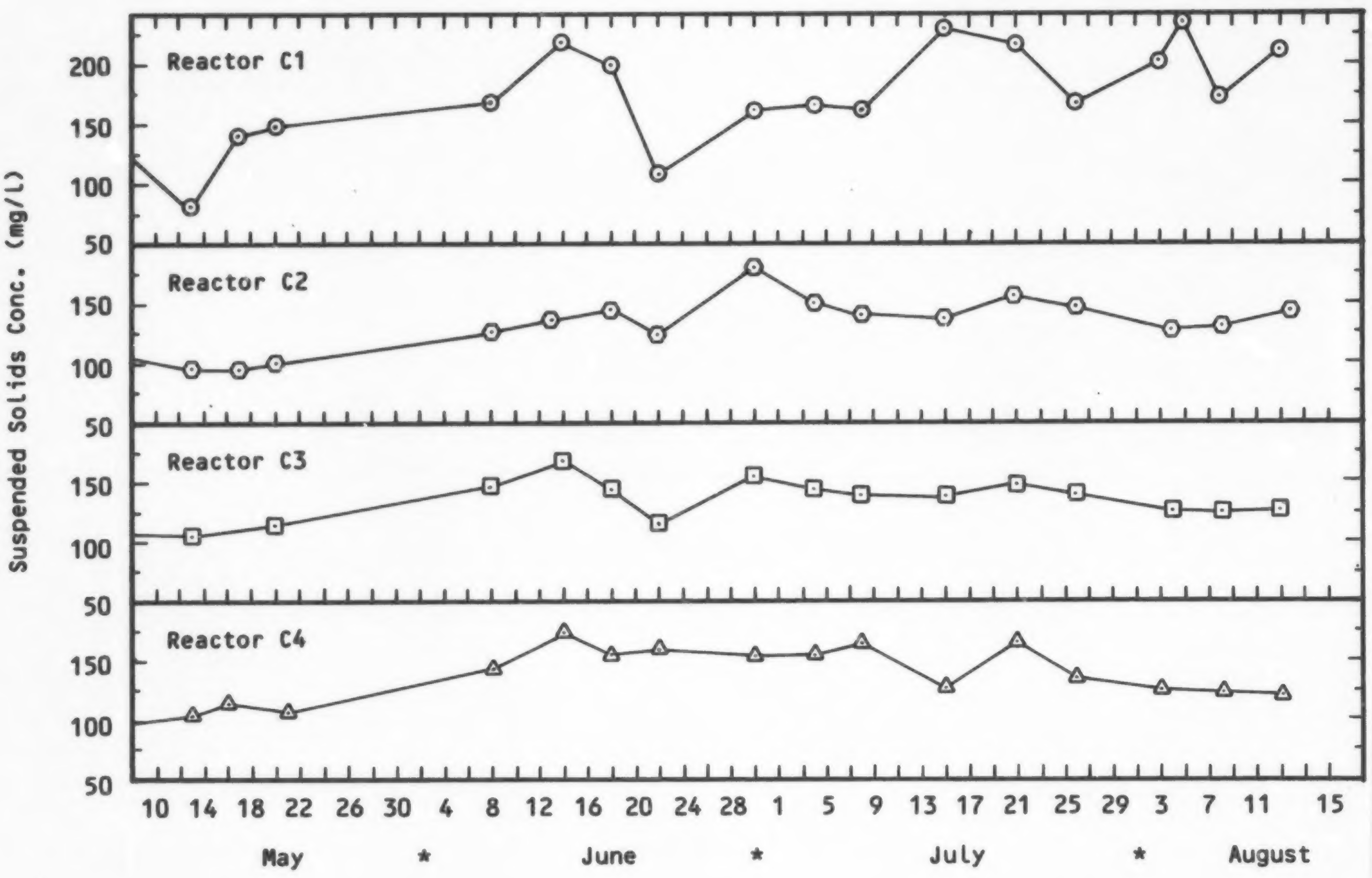

Figure 18 Suspended solids concentration in control reactors during Phase II testing.

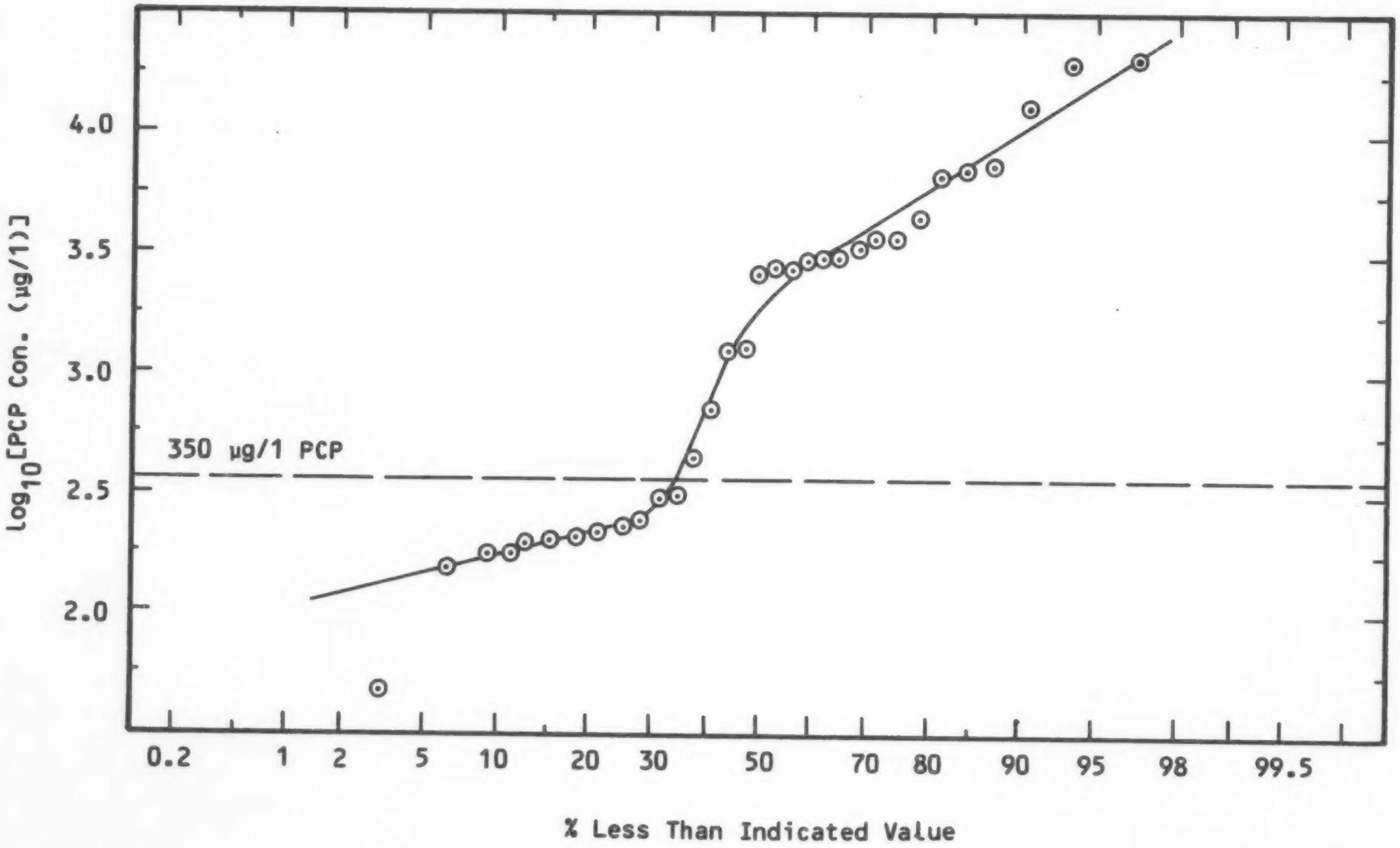

Figure 19 Probability plot of PCP concentration in test reactor I (SRT=3 days).

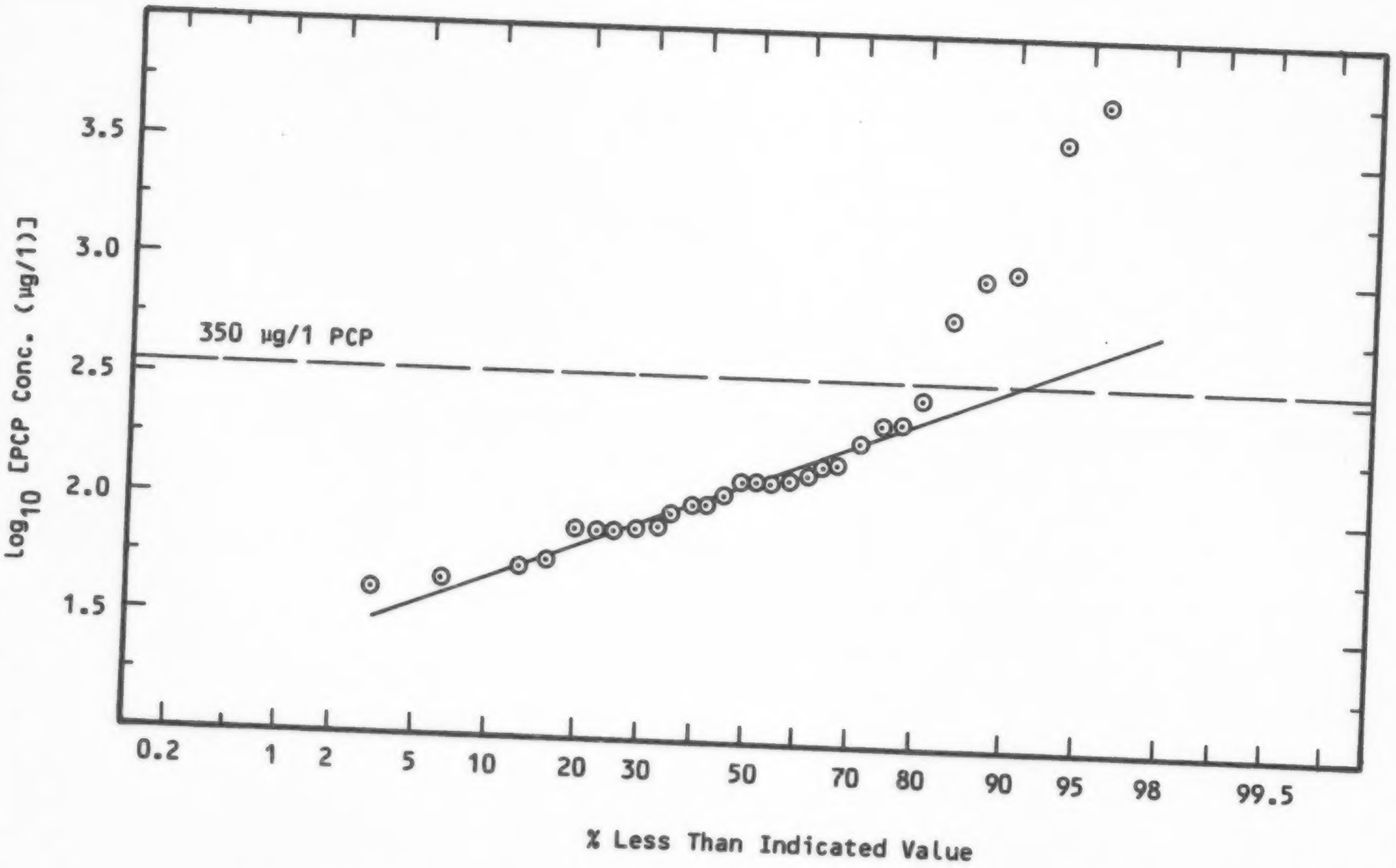

Figure 20 Probability plot of PCP concentration in test reactor 2 (SRT=7 days).

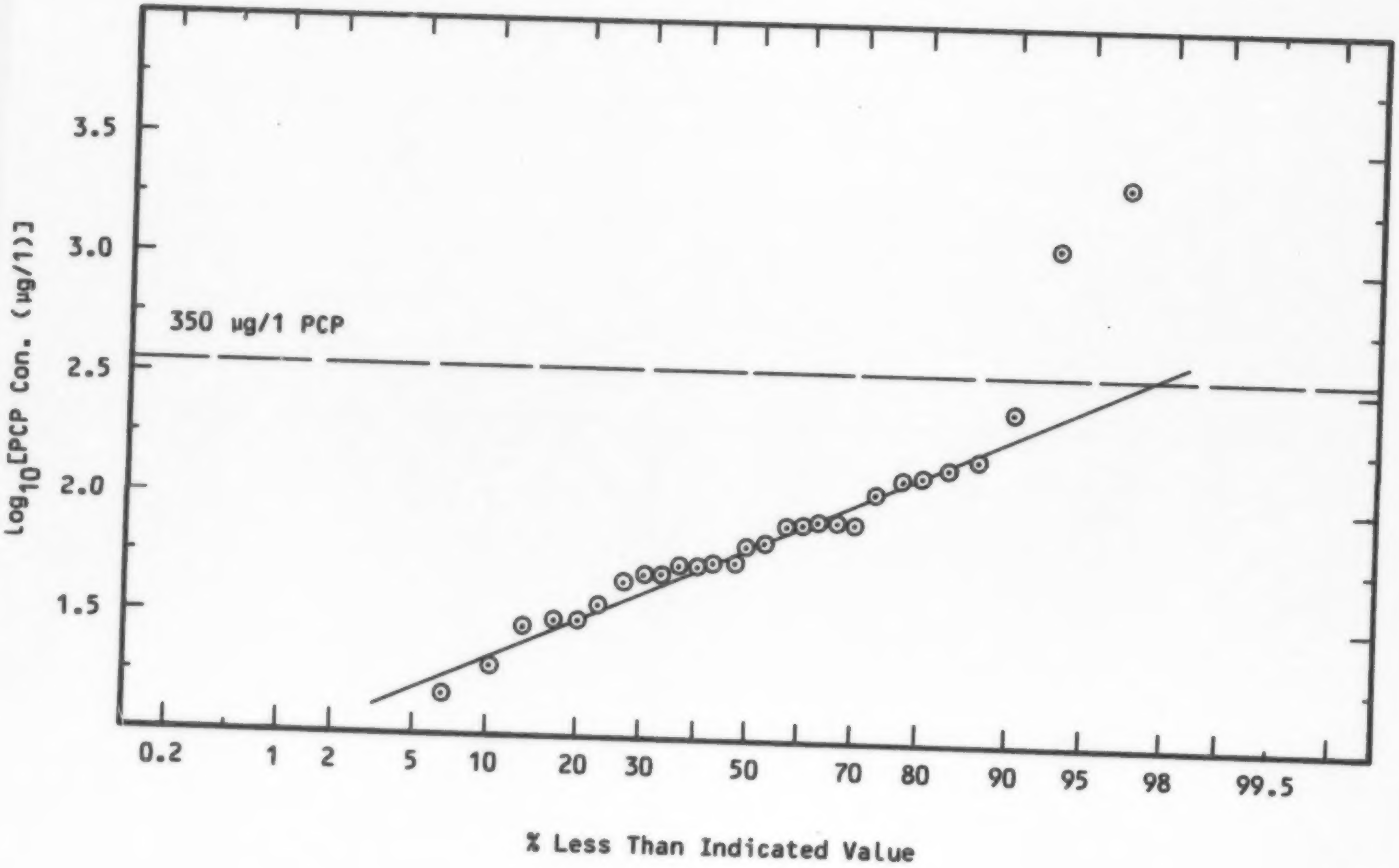

Figure 21 Probability plot of PCP concentration in test reactor 3 (SRT = 11 days).

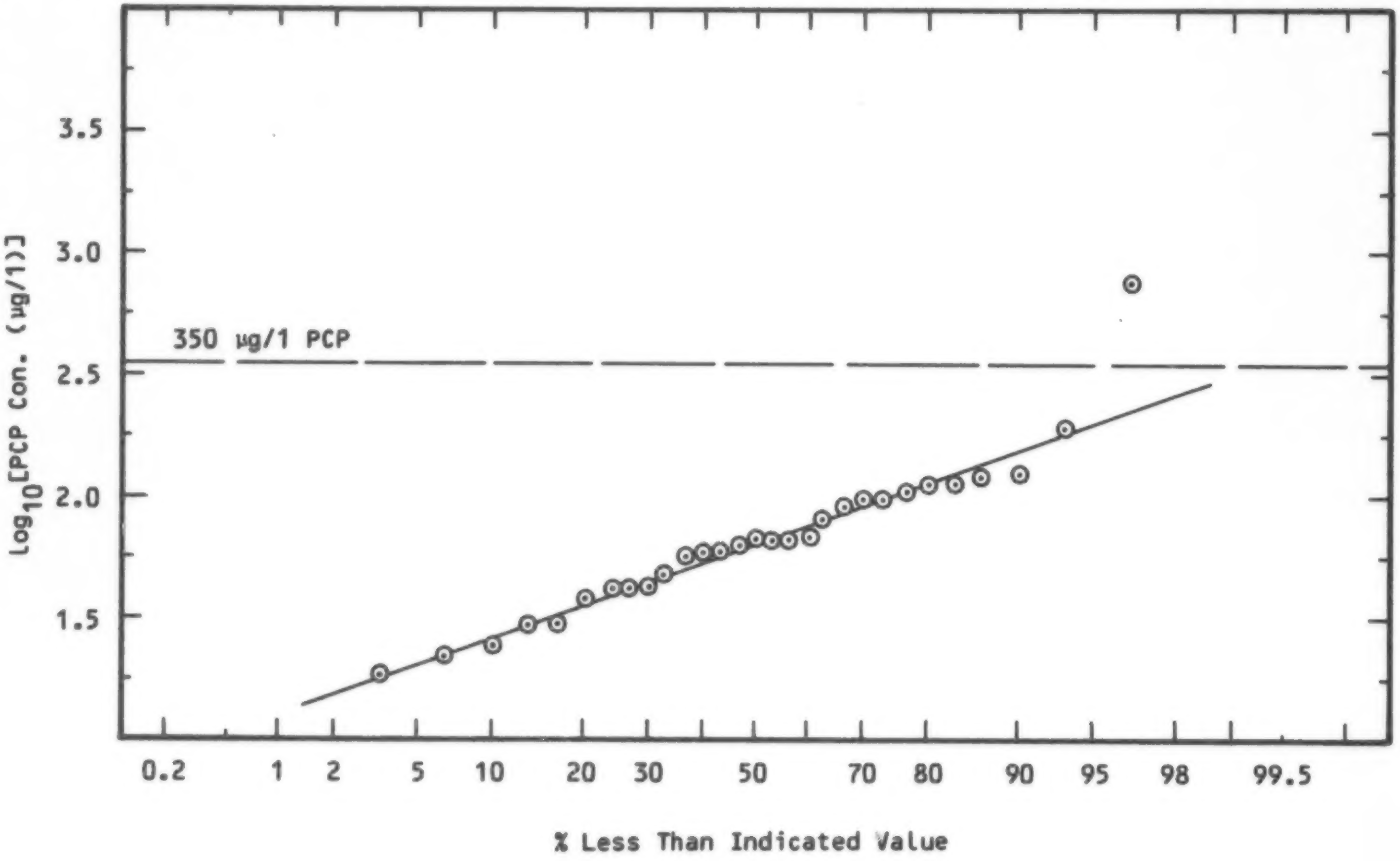

Figure 22 Probability plot of PCP concentration in test reactor 4 (SRT = 15 days).

concentration values and to estimate the standard deviation of the distributions. The 95% confidence limits of the mean values were calculated using the student's t distribution according to the following equation:

$$\langle\mu\rangle_{1-\alpha} = [\bar{X} - t_{\alpha/2,\ n-1} \cdot \sigma/\sqrt{n}\ ;\ \bar{X} + t_{\alpha/2,\ n-1}\ \sigma/\sqrt{n}] \tag{18}$$

where:

$\alpha = (1 - 0.95) = 0.05$

n = number of data points

σ = log standard deviation of population

$\bar{X}$ = log mean value

$t_{\alpha/2,\ n-1}$ = percentile value of the t distribution with (n-1) degrees of freedom

These calculated values appear in Table 11 and were used in subsequent evaluation of the data.

Evaluation of Kinetic Relationships for PCP Removal--

A stated objective of this study was to obtain biodegradation data in such a way that it could be used to predict the fate of the priority pollutant in an aerobic wastewater treatment system. Cursory examination of the data collected clearly showed the effluent PCP concentration was related to the SRT. If kinetic constants were obtainable from these data, one should be able to predict the level of PCP in the effluent at any chosen SRT.

The question of the prediction of the concentration of a single component in a multicomponent substrate is one that has received little attention and is one which needs much study. The literature in the area is rather limited and can tend to be rather complicated, depending upon the situation. Believing that a simple and straightforward approach is the most practical when it works, it was decided to try a simple noninteractive model (6) to allow prediction of the PCP level. Basically, such a model assumes that only a portion of the biomass is contributing to the removal of PCP and that there is a functional relationship between the specific growth rate of that biomass and the PCP concentration in the reactor. As pointed out earlier, the most widely utilized functional relationship is that of Monod:

$$\mu = \frac{\mu_m S}{K_s + S} \tag{4}$$

where: μ = Specific growth rate of the biomass (T^{-1}).

μ_m = Maximum specific growth rate which the biomass is capable of achieving (T^{-1}).

K_s = Saturation constant for the particular compound under consideration, (M/L^3).

Table 11 Mean Values and 95% Confidence Intervals for Steady State Operation

Reactor	Nominal SRT (days)	n	σ	t	95% Confidence Interval (logs converted to concentration units) mean	lower limit	upper limit
COD data					mg/l	mg/l	mg/l
R1	3	22	0.16	2.08	30.2	25.7	35.6
R2	7	21	0.11	2.09	19.5	17.4	21.9
R3	11	22	0.09	2.08	17.8	16.2	19.5
R4	15	22	0.08	2.08	21.9	20.2	23.7
C1	3	16	0.15	2.13	37.2	30.9	44.7
C2	7	22	0.13	2.08	21.9	19.1	25.1
C3	11	22	0.12	2.08	17.4	15.4	19.6
C4	15	22	0.11	2.08	15.9	14.2	17.7
PCP data					μg/l	μg/l	μg/l
R1	3	6	0.13	2.57	214	156	293
R2	7	19	0.22	2.10	105	82	134
R3	11	19	0.19	2.10	67.6	54.8	83.5
R4	15	24	0.24	2.07	64.6	51.1	81.5
Suspended Solids data					mg/l	mg/l	mg/l
R1	3	15	0.067	2.15	173	159	184
R2	7	15	0.051	2.15	158	148	169
R3	11	15	0.060	2.15	165	153	178
R4	15	15	0.068	2.15	130	119	142
C1	3	15	0.057	2.15	191	178	206
C2	7	14	0.053	2.16	141	132	152
C3	11	16	0.057	2.13	134	125	144
C4	15	15	0.057	2.15	145	135	156
MBAS data					mg/l	mg/l	mg/l
R1	3	17	0.180	1.746	0.60	0.50	0.72
R2	7	17	0.185	1.746	0.40	0.33	0.48
R3	11	14	0.187	1.771	0.30	0.24	0.37
R4	15	13	0.184	1.782	0.28	0.23	0.35
C1	3	14	0.181	1.771	0.78	0.64	0.95
C2	7	15	0.135	1.761	0.52	0.45	0.52
C3	11	15	0.177	1.761	0.37	0.31	0.45
C4	15	15	0.143	1.761	0.30	0.26	0.35

S = Concentration of the particular compound in the medium surrounding the organisms (ML^{-3}).

When the value of S is much lower than K_s the Monod equation can be simplified into a first-order relationship. The data in Table 11 show that the mean PCP concentration in the reactors was less than 220 μg/l. It seems likely that S is much lower than K_s, thusthe simplified form may be used:

$$\mu = (\mu_m/K_s)\,S \tag{19}$$

The specific growth rate, μ, of a microbial culture in a CSTR is often taken to be

$$\mu = (1/SRT) + d_s \tag{5}$$

In this expression the reciprocal of the SRT accounts for the loss of cells by removal from the reactor. The term d_s represents their loss by "decay", which is a collective term representing the loss by predation by higher life forms, death and lysis, as well as the decrease in mass associated with maintenance energy needs. As long as the biomass is homogeneously mixed, it is reasonable to believe that the organisms responsible for PCP degradation will be lost from the reactor at the same rate as all of the other organisms. Thus the term 1/SRT should be indicative of their loss. The similarity of d_s for the PCP-degrading biomass to that of the biomass as a whole, however, is something that is unknown. Thus, for the time being it must be assumed that d_s has a value characteristic of the PCP-degrading biomass alone.

A simple equation relating the reactor PCP concentration to the reactor SRT can be obtained by combining Eqs. (5) and (19):

$$(\mu_m/K_s)\,S = (1/SRT) + d_s \tag{20}$$

Rearranging this equation gives

$$S = (K_s/\mu_m)(1/SRT) + K_s d_s/\mu_m \tag{21}$$

Thus, a plot of the PCP concentration as a function of (1/SRT) should yield a straight line with a slope equal to (K_s/μ_m) and an ordinate intercept of $(K_s/\mu_m)d_s$. Consequently, the value of d_s and the lumped value of (K_s/μ_m) can be found from such a plot.

Figure 23 is a plot of the mean PCP concentration as a function of (1/SRT), where the SRT values used were the corrected ones given in Table 10. Examination of the Figure shows that an excellent fit was obtained (correlation coefficient = 0.998) which suggests that the use of the noninteractive model and the first-order approximation was justified. The slope of the line (K_s/μ_m) was 593 (μg·day)/liter and the intercept was 27 μg/l, giving a value of d_s of 0.046 day^{-1}.

A physical meaning can be attached to the value of the ordinate inter-

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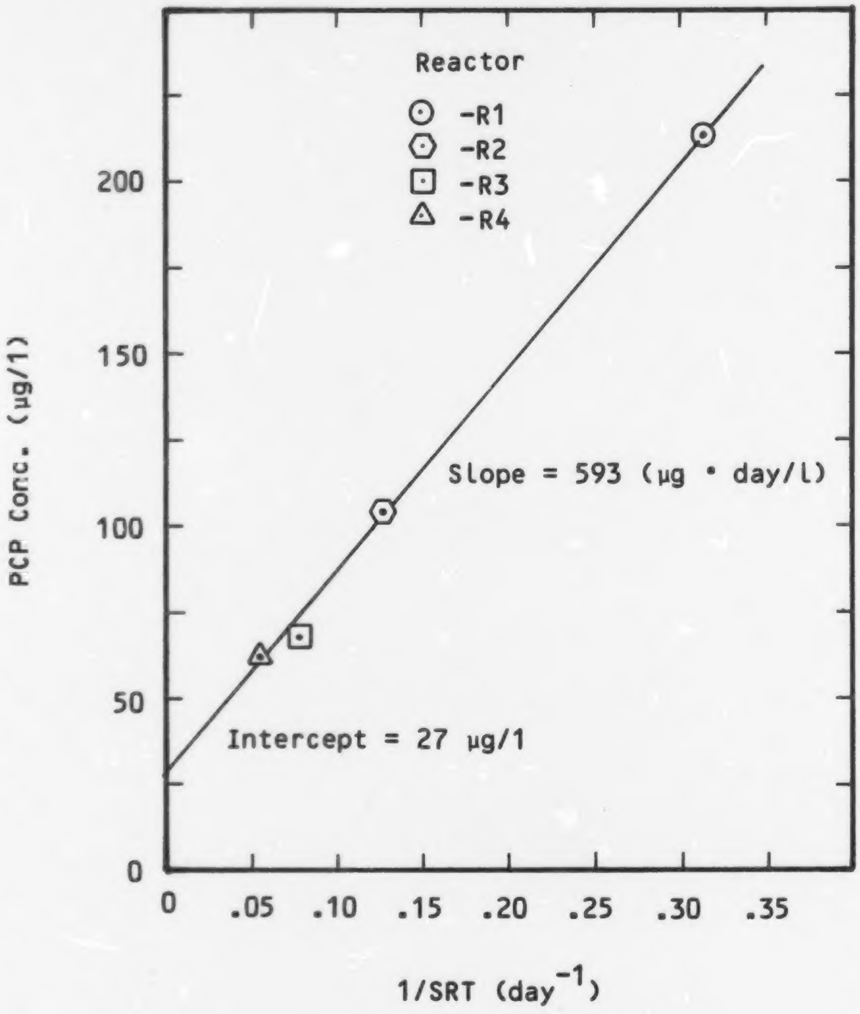

Figure 23 Plot of PCP concentration as a function of 1/SRT in accordance with Eq. 21.

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cept of the plot. This intercept occurs when 1/SRT is zero, a condition equivalent to a system with an infinite SRT. The substrate concentraion represented by this intercept is the minimum concentration possible in a single CSTR. It is determined by the decay rate of the cells since it is the substrate concentration required to drive the growth reaction at a rate just sufficient to balance the cells lost to decay. This concentration is 27 µg/l, as shown in Figure 23. This suggests that any system which uses a single complecely mixed reactor, such as completely mixed activated sludge, will be incapable of reducing the PCP concentration below 27 µg/l. The only way to achieve a lower PCP value would be to use a plug flow reactor or a configuration with reactors in series because in those reactors the specific growth rate is no longer controlled by the SRT alone.

As mentioned earlier, one question which arises when the degradation of a single component in a multicomponent mixture is being considered is whether the decay rate of the cells responsible for the degradation of that component is the same as the decay rate of the biomass as a whole. Information pertinent to that question can be obtained by estimating d from the SCOD data, which represents all of the organic matter, and comparing it to the d_s value from the PCP data. In this case, however, the approach used in Figure 23 cannot be employed because the SCOD values will include nonbiodegradable material whereas the S value in Eq. 21 must be for biodegradable material only. An alternative approach is to evaluate d from the relationship between the cell concentration and the reactor influent and effluent SCOD concentrations because in this way any nonbiodegradable SCOD is eliminated by the difference term (S_o-S):

$$X = \frac{Y_g(S_o-S)}{1 + d\ (SRT)} \qquad (22)$$

where: X = steady state cell concentration (ML^{-3})

d = decay constant for the biomass as a whole (T^{-1})

Y_g = true growth yield (M/M)

S_o = influent SCOD concentration (M/L^3)

S = effluent SCOD concentration (M/L^3)

This can be rearranged into a liner form, yielding:

$$(S_o-S)/X = 1/Y_g + (d/Y_g)(SRT) \qquad (23)$$

Therefore, a plot of $(S_o-S)/X$ versus SRT will yield a straight line with a slope equal to d/Y_g and an ordinate intercept equal to $1/Y_g$.

The suspended solids and SCOD data, taken during the operation of the reactors, may be used to estimate both Y_g and d. Before this is done, however, the effect of evaporation losses must be taken into account. This can be done by expressing the influent SCOD concentration as an effective influent concentration by assuming that the mass of substrate present in the 500 ml/day of influent was present in the actual effluent volume. This calculation is shown in Table 12.

Table 12 Effective Influent SCOD Concentrations

Reactor No.	Nominal SRT days	Concentration Factor	Effective Influent SCOD Controls* mg/l	Effective Influent SCOD Test** mg/l
1	3	500/470 = 1.06	456	498
2	7	500/450 = 1.11	477	529
3	11	500/430 = 1.16	499	545
4	15	500/410 = 1.22	525	573

*based on influent SCOD of 430 mg/l
**based on influent SCOD of 470 mg/l

Using these effective concentration as the S_o values, a plot of $(S_o-S)/S$ versus SRT was made, as shown in Figure 24. The corrected SRT values from Table 10 were used, as discussed earlier. The data from the control and test reactors formed two lines with slightly different slopes. However, an F-test of the data showed that the slopes were not significantly different. Therefore all eight data points were used to fit a single line. From the slope and intercept of this line, Y_g was determined to be 0.45 g cells/g SCOD removed and d was found to be 0.051 day^{-1}. Both values are in the range commonly seen in activated sludge systems treating organic matter similar to that employed in this research. More significantly, however, the value of d obtained from the SCOD data compares quite favorably with the value of d_s obtained from the PCP data. This suggests that the decay characteristics of the organisms responsible for PCP degradation were quite similar to those of the biomass as a whole. This, in turn, suggests that the specific growth rate of those organisms can be estimated from the SRT and decay rate for the major biomass in the system.

The use of a kinetic relationship like Eq. (4) to describe PCP degradation for the purpose of estimating the steady-state performance of a full-scale system should give an accurate prediction for reactors that approach the completely-mixed state because the organisms in such reactors all have the same average specific growth rate (determined by the SRT and decay rate for the system) and because Eq. (4) is based solely upon that growth rate. Furthermore, although no data have been collected to support the contention, it seems reasonable that the kinetic parameters for PCP degradation determined herein should be applicable to other PCP containing wastewaters which are otherwise "typically domestic" in their composition. Thus, unless a wastewater contains other components which adversely affect the PCP-degrading population in the activated sludge, it seems reasonable to expect removals down to approximately 100 μg/l in systems with normal SRT's. As pointed out earlier, however, the data also suggest that it will be difficult to reduce PCP to a concentration below approximately 30 μg/l in a system containing a single completely-mixed reactor.

It is important to realize that while volatilization and adsorption may

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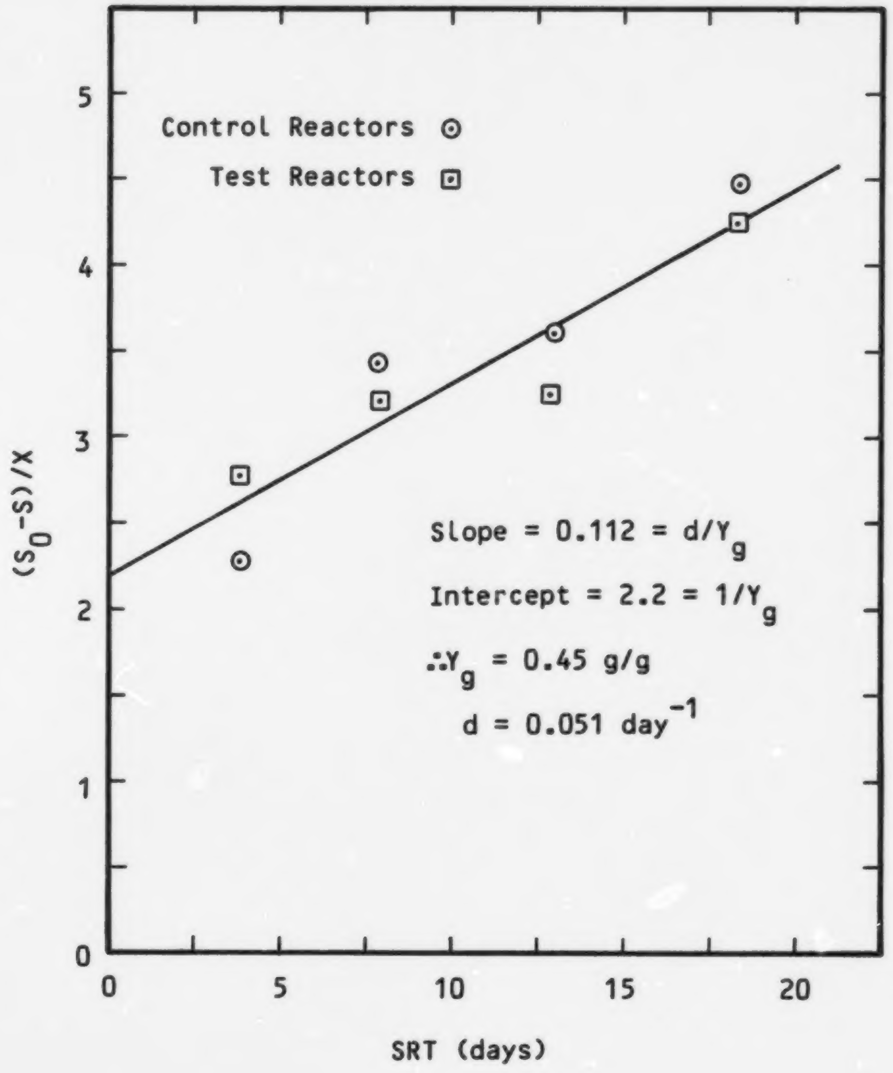

Figure 24 Plot for the determination of the true growth yield and the decay rate in accordance with Eq. 23.

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contribute to substrate removal, the steady state concentration of the priority pollutant remaining in solution will depend solely upon the specific growth rate imposed upon a completely-mixed reactor. As long as the above kinetic approach is used to determine that concentration, the removal by the other mechanisms need not be considered to determine the steady-state concentration. This is because the approach is based upon a relationship between specific growth rate and pollutant concentration rather than upon the specific substrate removal rate. If the latter approach were used, the contribution of the other mechanisms would have to be considered because they determine the mass of pollutant-degrading organisms in the system (Eq. 8). The simplicity of the specific growth rate approach makes solution of the problem possible in this situation because the growth rate is known. It is important to emphasize, however, that if the reactor cannot be approximated as being completely mixed, or as a series of completely mixed reactors, then this approach cannot be used and the contribution of the other mechanisms must be considered. That approach would require knowledge of the mass of PCP-degrading organisms in the biomass. While techniques can be proposed for measuring that mass (some have been tried in our lab) they are complicated and not very accurate. Thus, it appears that for the time being the approach proposed and used herein represents the most reasonable approximation of the level of performance that can be expected from an activated sludge system.

Although the contribution of stripping and adsorption need not be considered to estimate the steady-state pollutant concentration in a completely-mixed reactor, the amount of pollutant removed by each is an important consideration from both an ecological and regulatory viewpoint. Thus the contributions of each to PCP removal in the test reactors will be discussed later in this report.

Effect of PCP upon SCOD and MBAS Removal--

The effect which the presence of PCP has on other performance parameters of the system, such as mean SCOD and MBAS concentration, can be evaluated by a similar kinetic analysis.

Figure 25 shows a plot of mean SCOD concentration versus 1/SRT + d for the control reactors and the PCP test reactors. It can be seen that the data for the control reactors form a very good straight line. The data point corresponding to reactor R4, the point to the far left, is located far above the line formed by the other three points. This high SCOD concentration was noted previously and attributed to the dense, granular floc structure present in the reactor during this period. Consequently, the line was fitted through the other three points which were all from reactors with similar floc structure. The slope of the line for the test reactors is slightly smaller than the slope of the line for the control reactors. This suggests that the presence of PCP in the reactors may have accelerated the degradation of SCOD, possibly by its ability to uncouple oxidative phosphoryation. However, when a statistical F-test was run on the raw data to determine the level of significance, the data demonstrated less than the 75% confidence level. Thus one should not place too much emphasis upon the differences between the two plots.

A similar plot of mean MBAS concentration is shown in Figure 26. The

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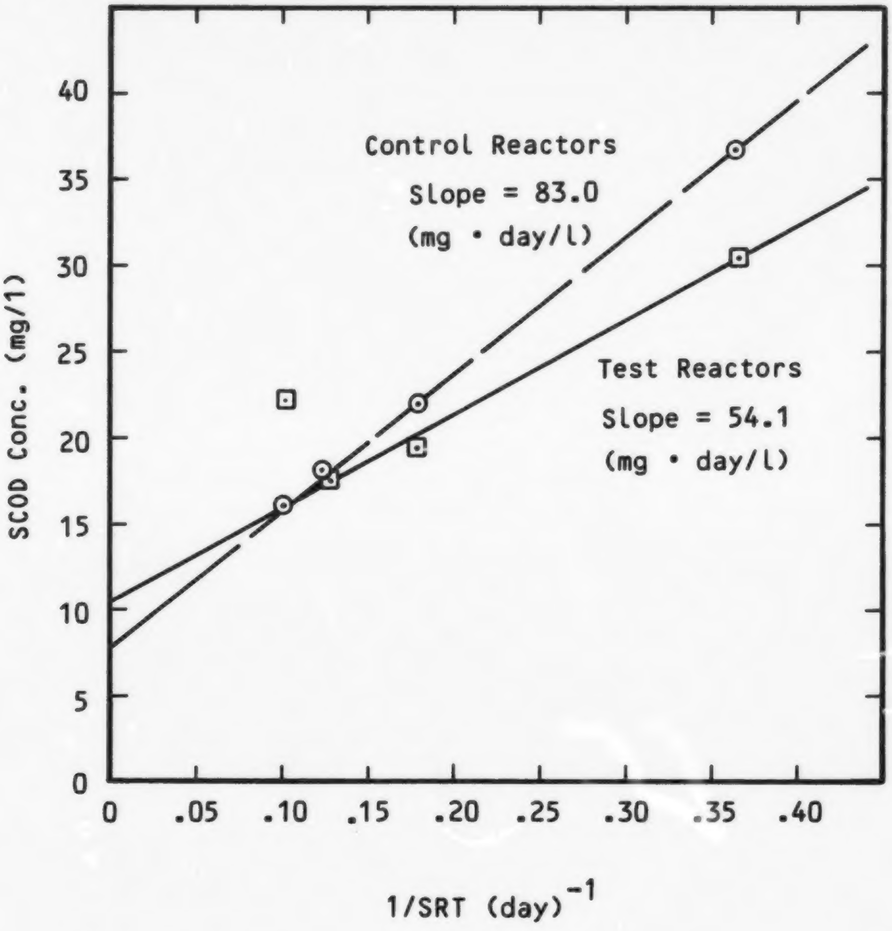

Figure 25 Plot of SCOD concentration as a function of 1/SRT in accordance with Eq. 21.

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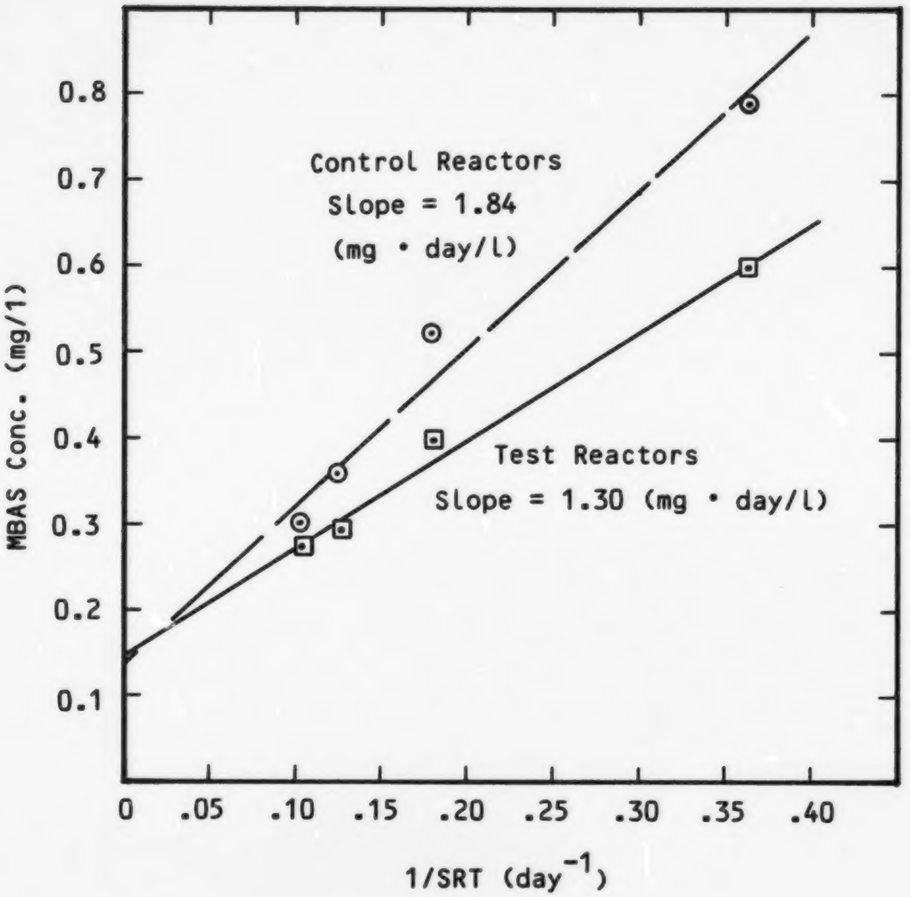

Figure 26 Plot of MBAS concentration as a function of 1/SRT in accordance with Eq. 21.

data for both the control reactors and the test reactors form highly correlated straight lines. As with the SCOD concentration, the slope of the line formed by the test reactor data is smaller than that formed by the controls. When an F-test was run on this data it was determined that the slopes were significantly different only to a level of confidence of 75%, indicating that PCP had little effect of MBAS degradation.

Correlation Between PCP and SCOD; PCP and MBAS--

One of the objectives of the protocol evaluation was to determine if the effluent PCP concentration could be correlated to other plant performance parameters. The first attempt to do this was to relate the effluent PCP concentration to the effluent SCOD concentration by plotting the PCP concentration versus the SCOD concentration on the same day. Figure 27 shows these plots. In each case the data are widely scattered along an almost vertical line, indicating that there was little or no correlation between the day-to-day SCOD and PCP concentrations because the PCP varied greatly while the SCOD changed relatively little. The plots for reactors R1 and R2 (the 3 and 7 day SRT test reactors) do show a slight degree of correlation, but this was due primarily to a a few data points taken during periods of major upsets when both the PCP and SCOD concentrations were high.

When a comparison was made between the mean PCP and SCOD values, on the other hand, it was seen that both parameters decreased with increasing SRT and therefore, a plot of the mean values (Figure 28) showed a high degree of correlation between them. This correlation was expected because concentrations of both COD and PCP are correlated to the SRT. It should be realized, however, that there will probably be a great deal of variation in PCP concentration occurring from day to day. The nature of the correlation will be specific for a given situation. For example, the nonbiodegradable SCOD concentration determines the intersection of the line with the ordinate axis and thus will locate the line in a horizontal direction. Furthermore, the ease with which the organic matter in the sewage is degraded will influence the slope of the line.

One possible reason that no usable correlation existed between the daily PCP and SCOD concentrations was that the former represented a specific organic compound whereas the latter represented the sum of all organic compounds in the effluent, both degradable and nondegradable. Thus, it was thought that a better correlation might exist between the PCP concentration and the concentration of a more narrowly defined group of compounds. MBAS was chosen as a candidate group because it was thought that its more recalcitrant nature would more closely mimic the degradation of the test compound. Figure 29 shows plots of PCP concentration versus the MBAS concentration measured on the same day. There was no significant correlation between the daily concentrations of PCP and MBAS. A plot of the mean PCP concentration versus the mean MBAS concentration yielded a highly correlated line (Figure 30). This correlation was expected because concentrations of both compounds are correlated to the SRT.

As with the SCOD data, the correlation between the mean values suggests that, when averaged over a long period, the effluent MBAS concentrations can

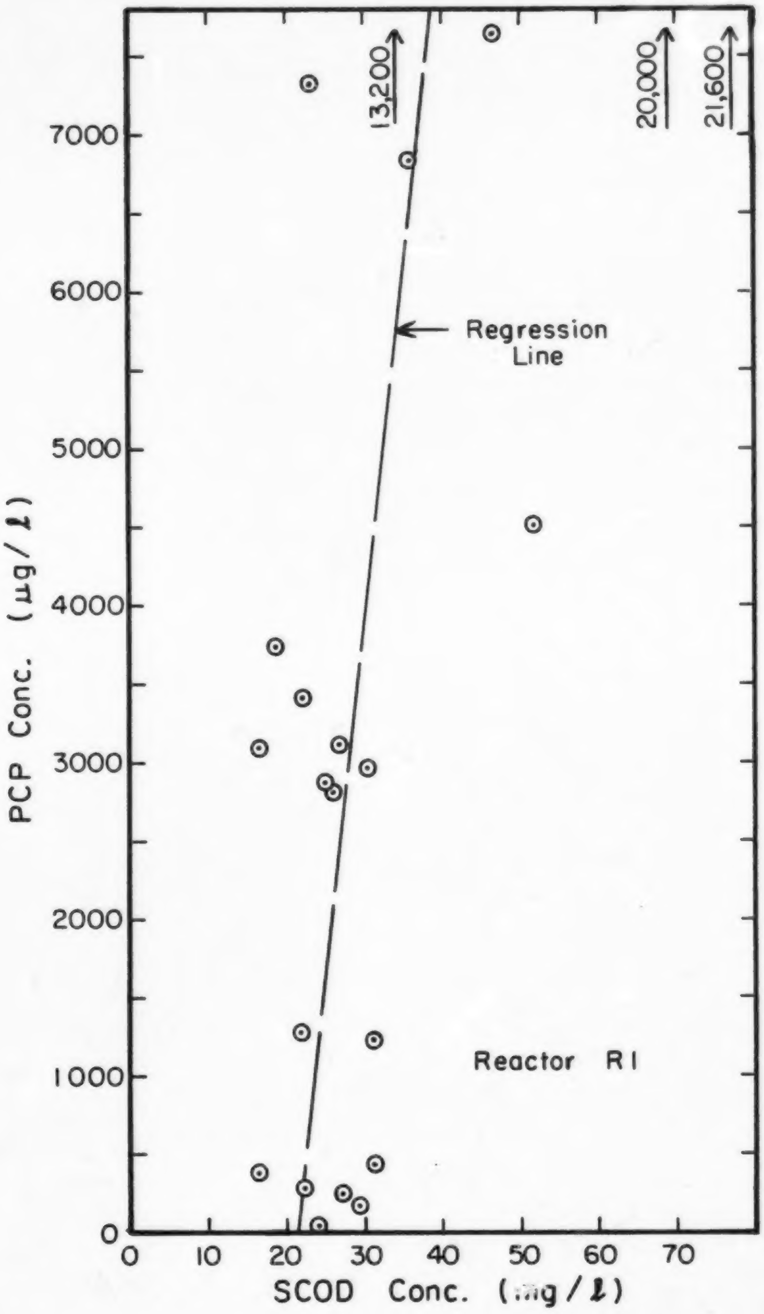

Figure 27a Correlation of PCP concentration with SCOD concentration in test reactor 1.

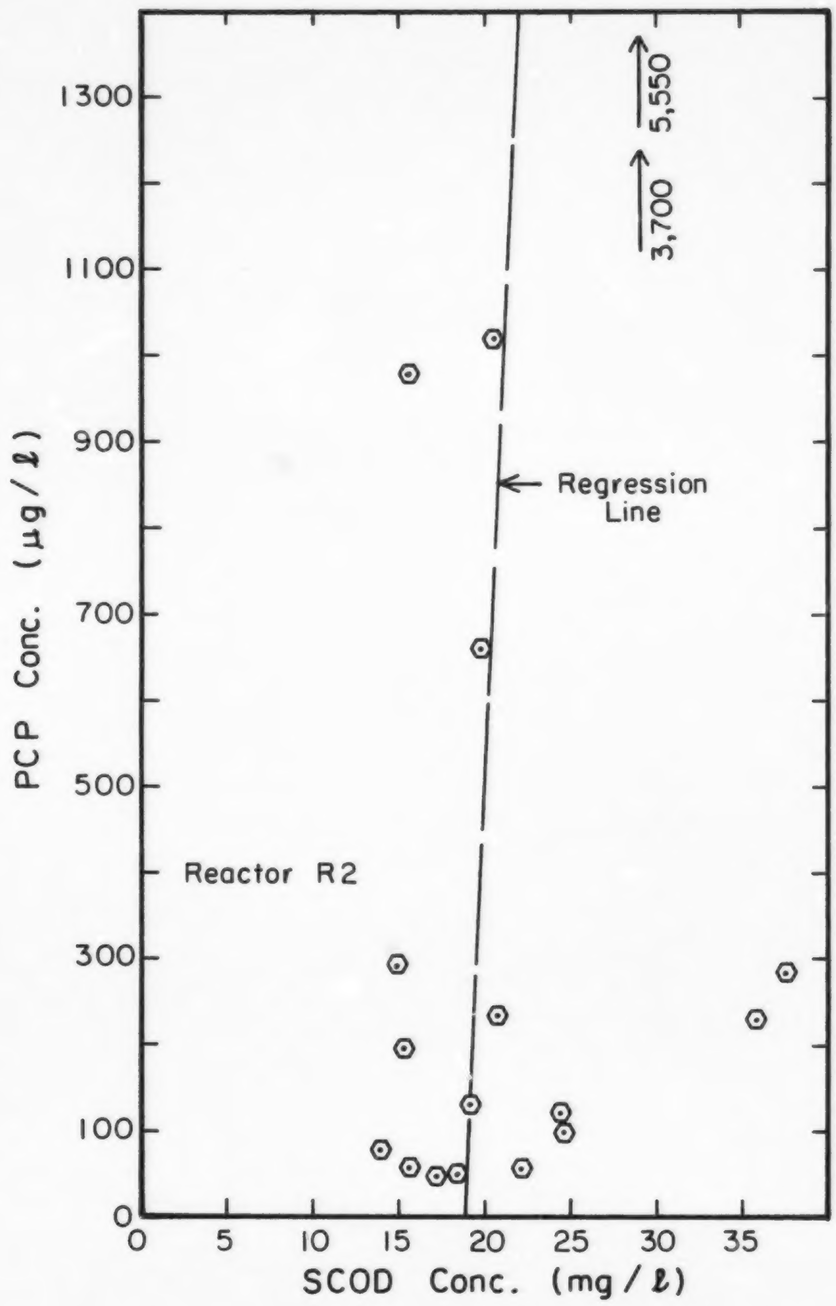

Figure 27b Correlation of PCP concentration with SCOD concentration in test reactor 2.

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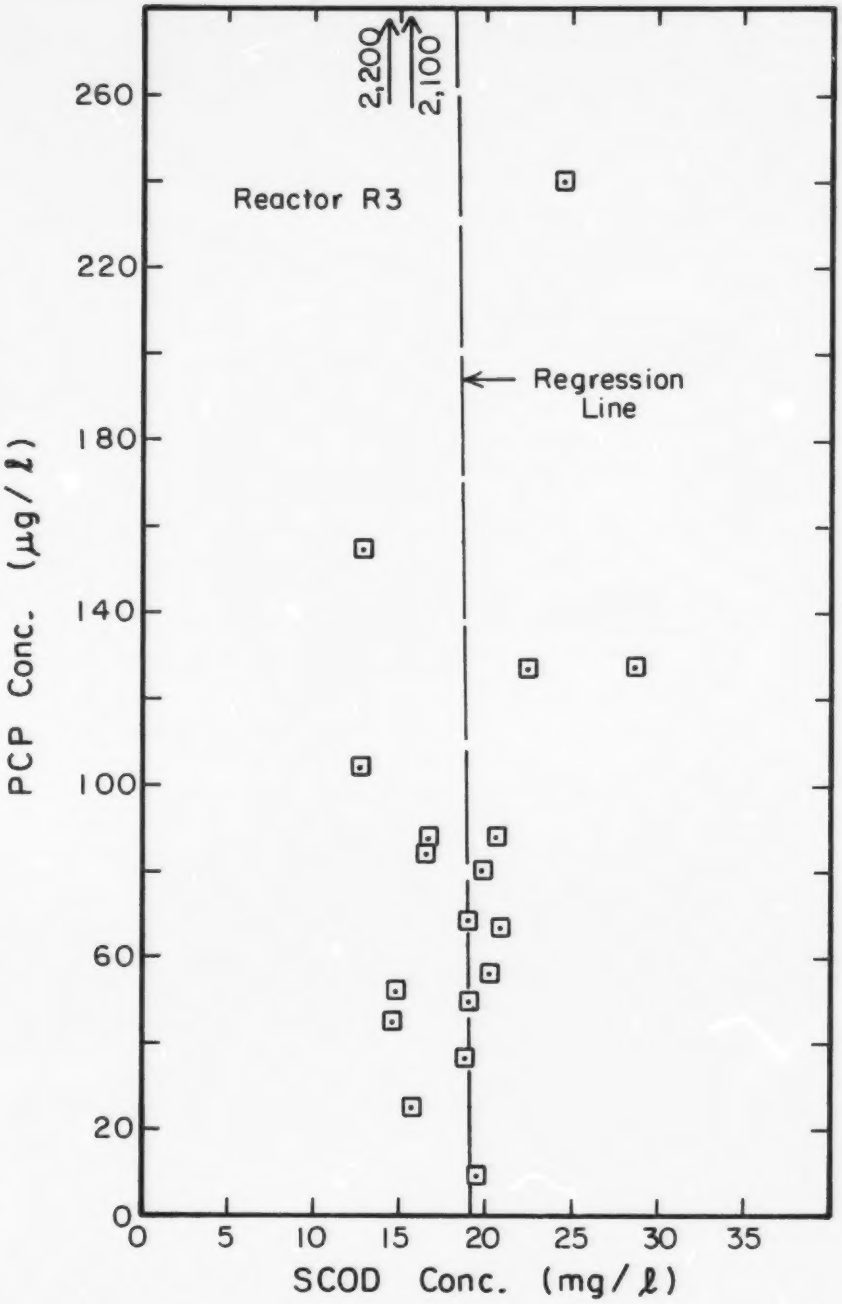

Figure 27c Correlation of PCP concentration with SCOD concentration in test reactor 3.

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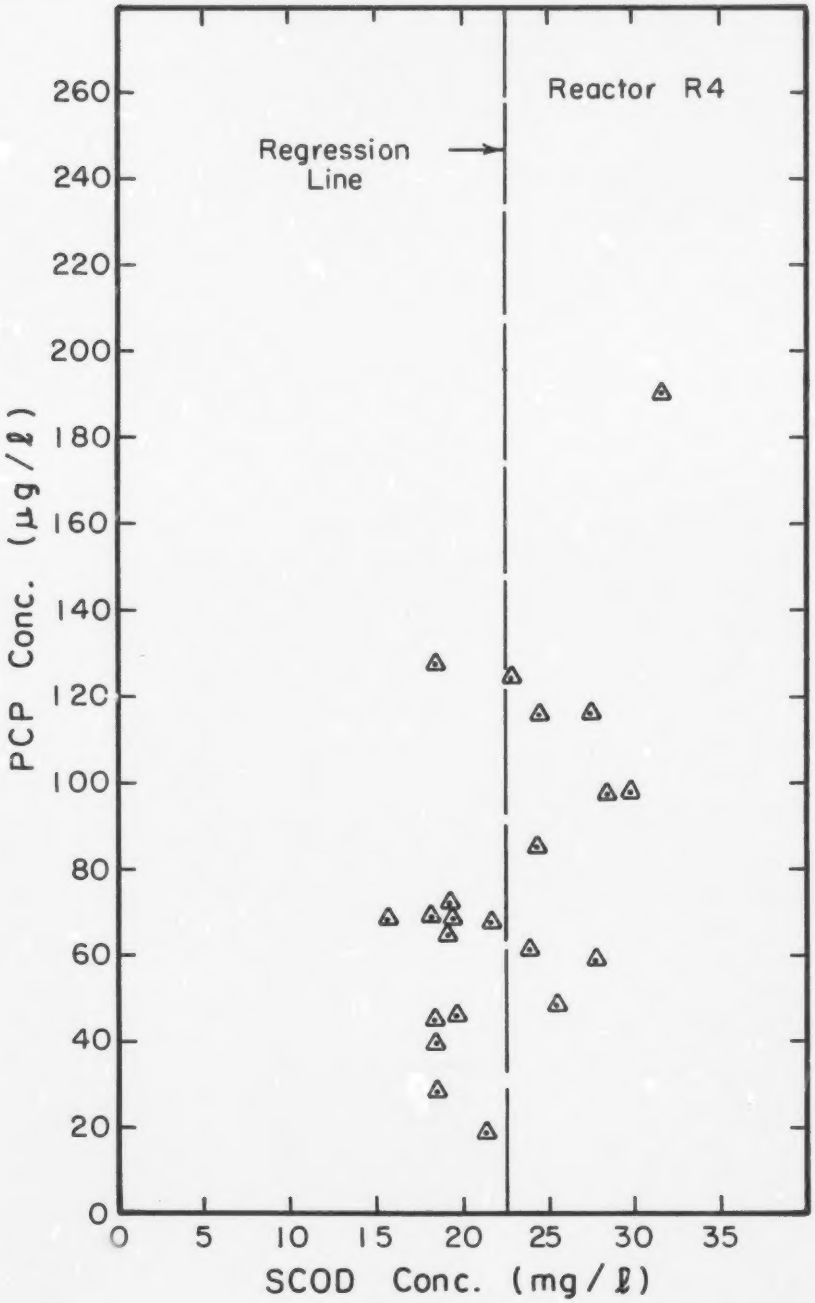

Figure 27d Correlation of PCP concentration with SCOD concentration in test reactor 4.

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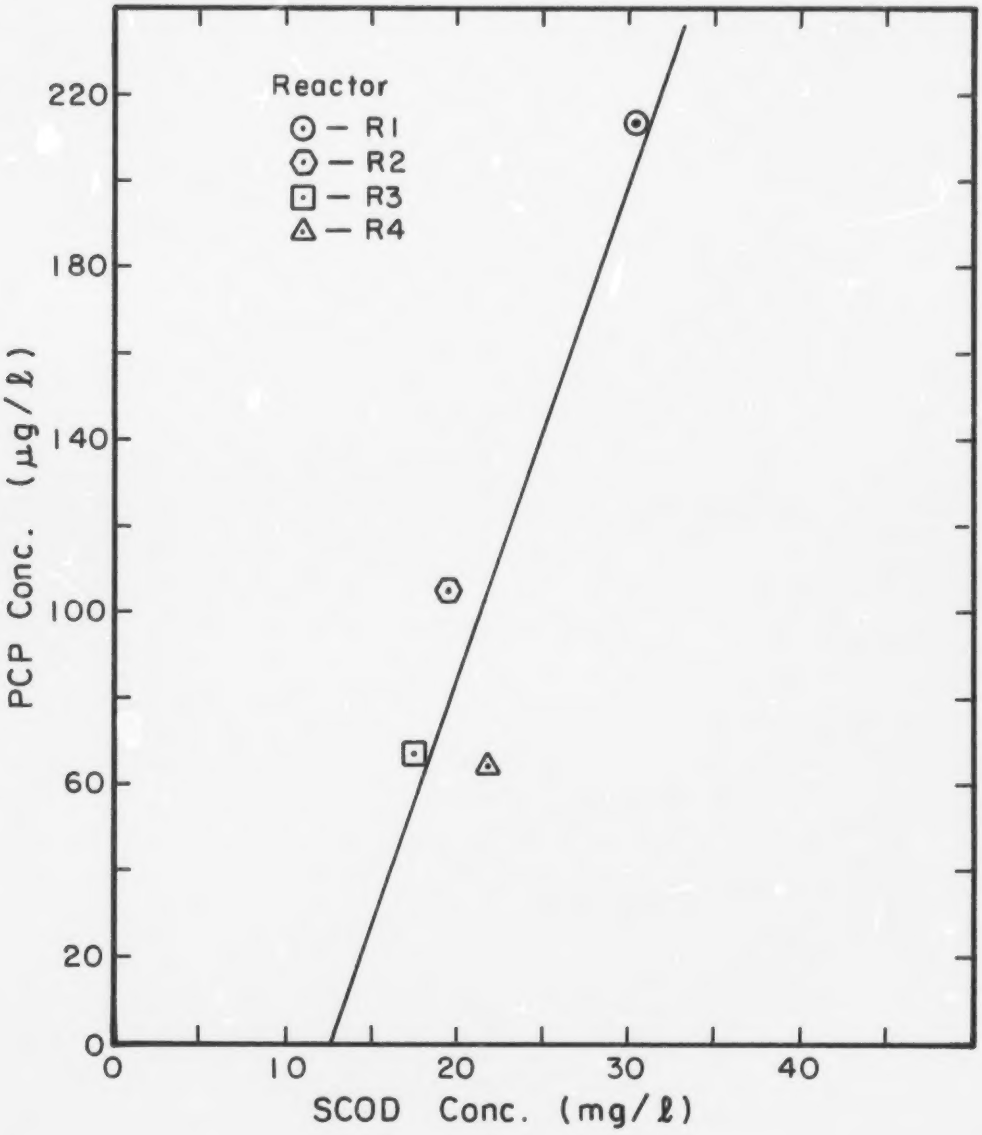

Figure 28 Correlation of mean PCP concentration with mean SCOD concentration in the test reactor.

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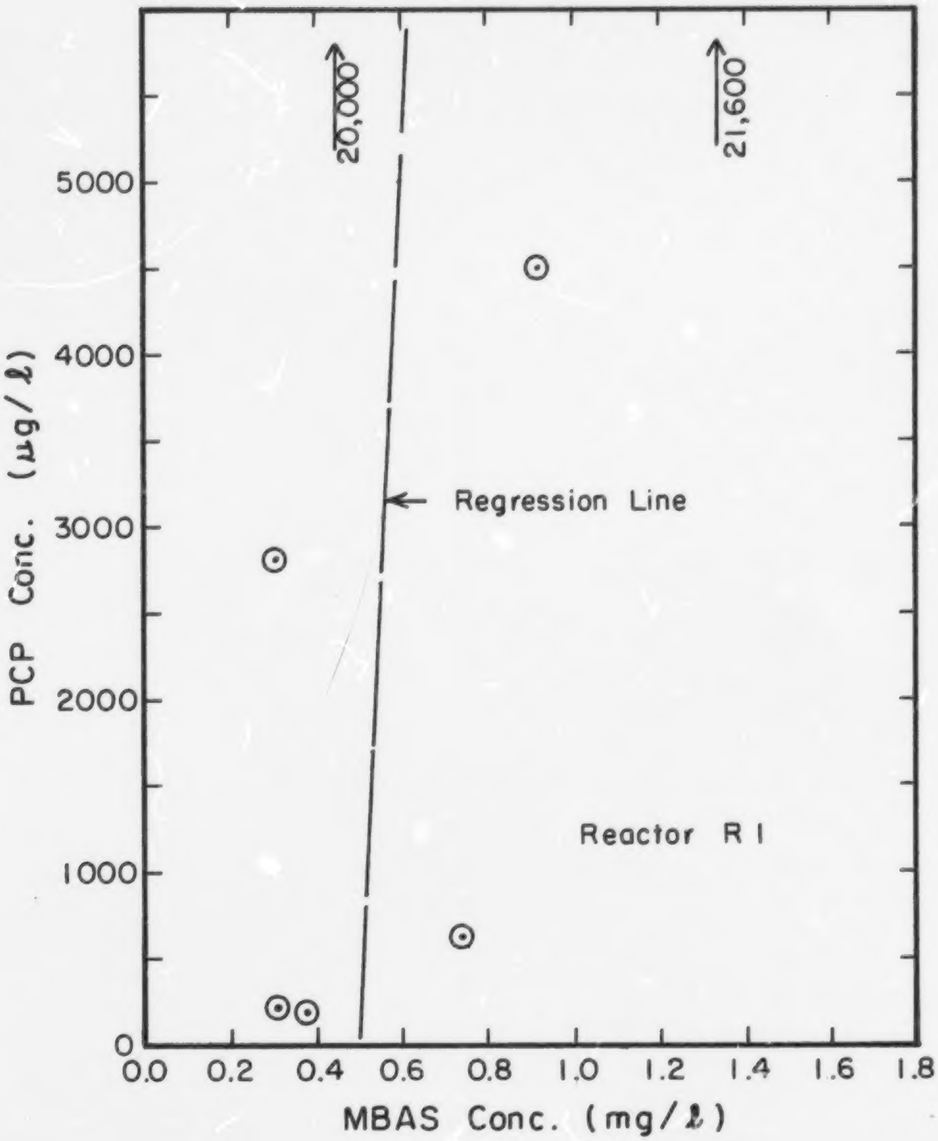

Figure 29a Correlation of PCP concentration with MBAS concentration in test reactor 1.

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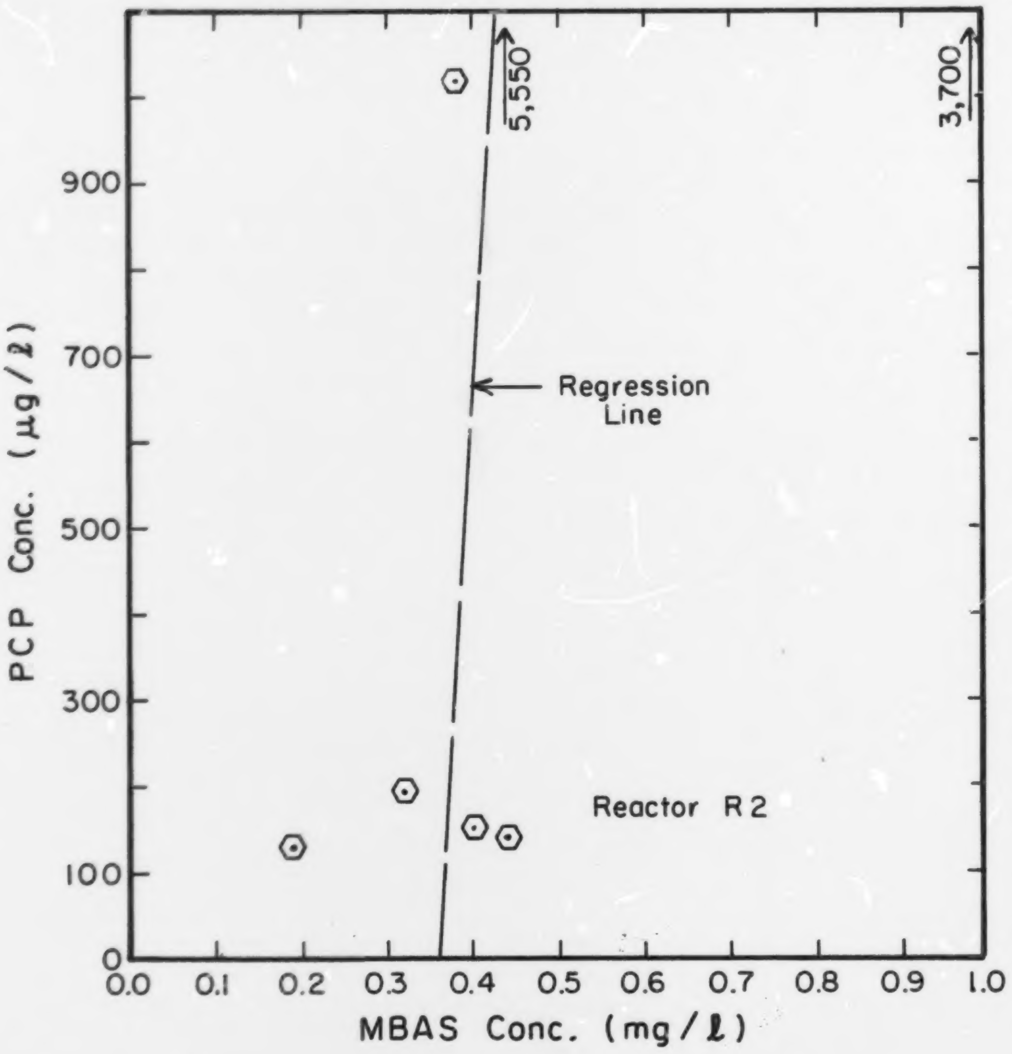

Figure 29b Correlation of PCP concentration with MBAS concentration in test reactor 2.

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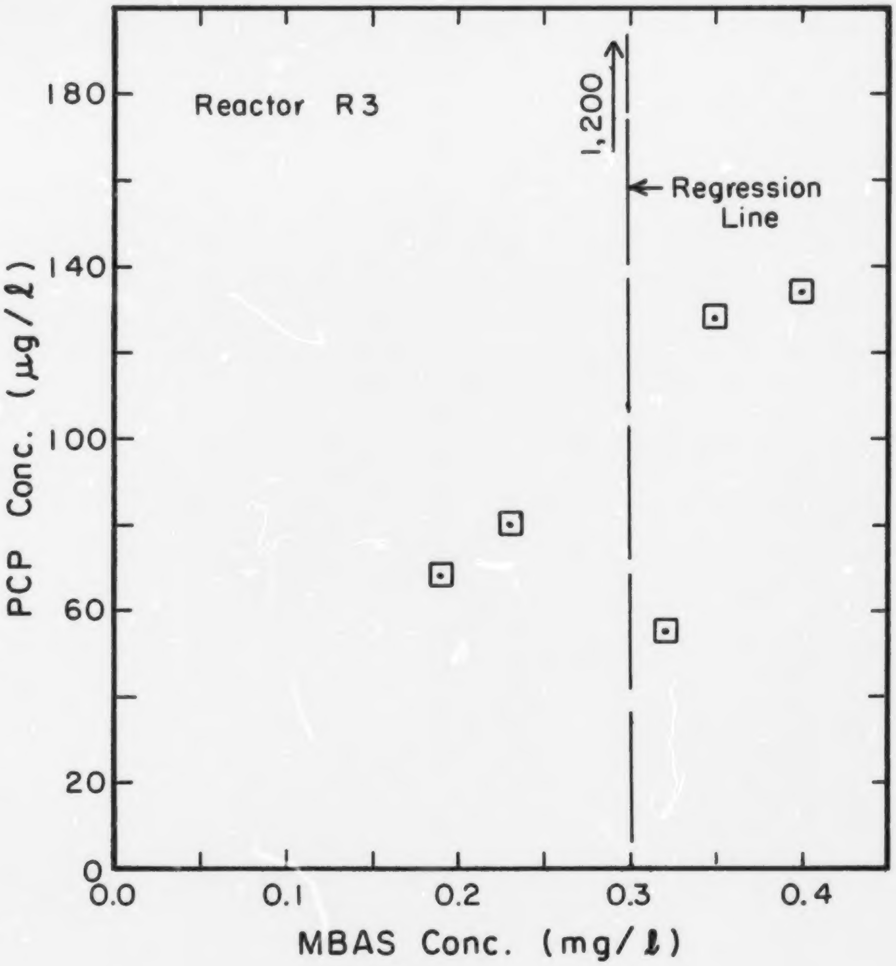

Figure 29c Correlation of PCP concentration with MBAS concentration in test reactor 3.

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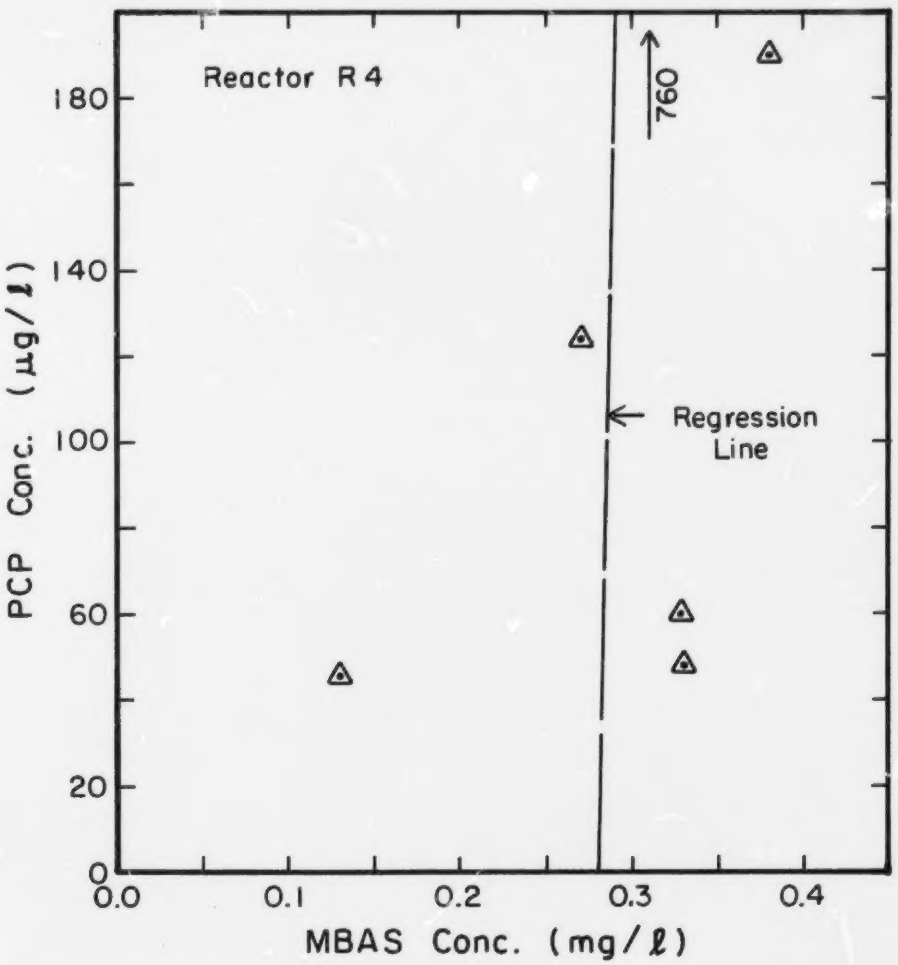

Figure 29d Correlation of PCP concentration with MBAS concentration in test reactor 4.

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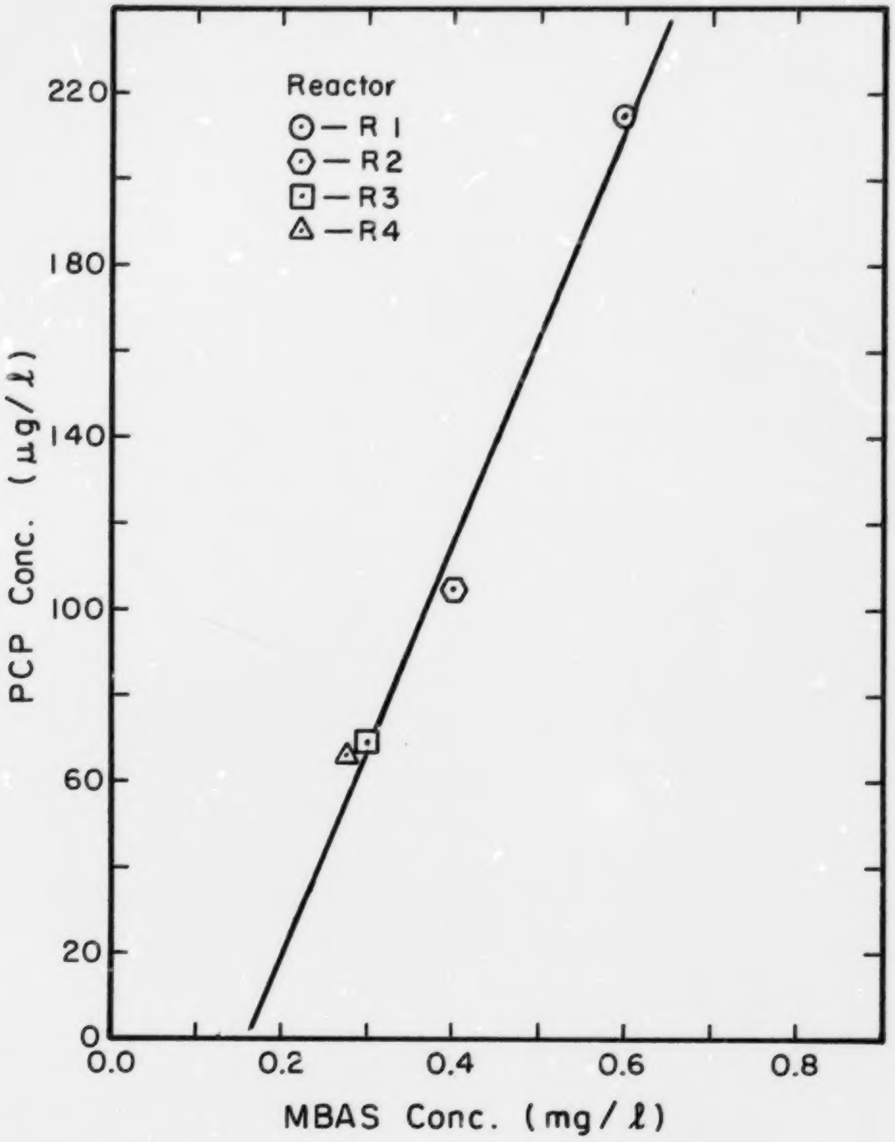

Figure 30 Correlation of mean PCP concentration with mean MBAS concentration in the test reactor.

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predict the average PCP concentrations. Although the MBAS analysis measures a more narrowly defined group of organics than SCOD, it is still a nonspecific analysis and thus the relationship between it and the PCP concentration is subject to the same factors that affected the SCOD-PCP relationship; that is, the presence of nondegradable MBAS and the presence of many different compounds which react with methylene blue, each of which has its own ease of degradation. Consequently, the use of an internal standard to predict performance of the treatment system for a priority pollutant seems unwarranted and subsequent use of MBAS for this purpose has been discontinued.

Evaluation of the Effect of PCP Inhibition--

It was hypothesized that the variability of the PCP concentration data, particularly at short SRT's, was related to the toxic properties of PCP on bacterial cells responsible for its degradation. Experiments were designed to provide some additional insight into the cause-effect relationship despite the fact that the original test protocol did not specify such experiments. PCP inhibition was suggested by the log-normal probability plots of the PCP data, particularly from reactor R1 (SRT = 3 day). As shown in Figure 19, two log-normally distributed data populations appeared, the second distribution becoming apparent above 350 μg PCP/l. Furthermore, the data from the other reactors also deviated from the log-normal distribution whenever the PCP concentration exceeded 350 μg/l (see Figures 20-22). All of these data support the hypothesis that above this level of PCP the degradation rate is reduced and the PCP concentration increases. This phenomenon has been described as substrate inhibition and leads to the kinetic anomaly by which two steady states can exist for a single SRT; each steady state being characterized by either a low, noninhibiting, concentration of substrate or by a high, inhibiting, concentration of substrate. A theoretical graphic representation of the anomaly is given in Figure 31.

To determine if substrate inhibition was affecting the degradation of PCP, the following experiment was performed. Four reaction flasks were prepared by diluting a concentrated slurry of test reactor cells with filtered control reactor effluent to give suspended solids concentrations of 50, 50, 300, and 3000 mg/l. The two flasks containing 50 mg/l cells were spiked with 100 and 500 μg/l PCP. The flask with 300 mg/l cells was spiked to 2000 μg/l PCP while the final flask was spiked to 12,000 μg/l PCP. These solids concentrations were chosen to yield an approximately constant percent removal of PCP per hour for each of the flasks using assumed specific degradation rates similar to the rates in the CSTR reactors. The assumed rates reflected an expected strongly inhibitory effect of PCP concentrations over 1000 μg/l, as reported in the literature. Before the solids used in the 300 mg/l and 3000 mg/l flasks were diluted and spiked with PCP, they were put in effluent containing 2000 μg/l and 12,000 μg/l PCP respectively, for 1 hour. They were then removed from this liquid by centrifugation and resuspended in the test media. This step was done to minimize the effect of adsorption of PCP onto the biomass by allowing PCP to adsorb onto the solids and to equilibrate at 2000 and 12,000 μg/l before the cells were added to the test media. Without this step, PCP removal by adsorption would appear to be due to biodegradation and would obscure the rate of PCP biodegradation. Because the amount of adsorption likely to occur in the flasks containing 50 mg/l of

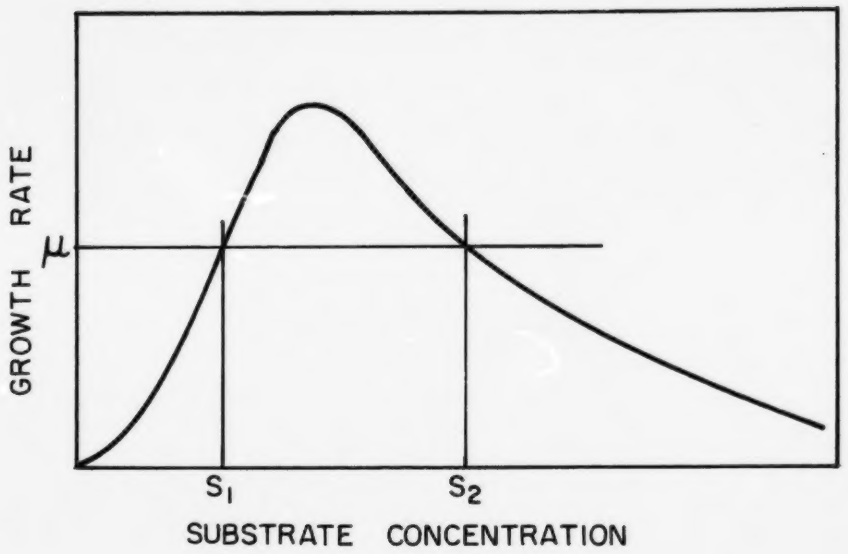

Figure 31 Schematic representation of substrate inhibition kinetics.

cells and low concentrations of PCP was considered to be negligible, no such correction was made for those flasks. The PCP present in the liquid phase of the test media was then determined at various times in each of the flasks for 12 hours. The results of this analysis are shown in Figure 32 where smooth curves were drawn by eye to closely match the data points. The dashed lines in this figure represent a constant specific PCP removal rate of 500 µg/hr·g cells, and are provided to allow comparisons between graphs with different magnitude scales.

The initial concentration of PCP (100 µg/l versus 500 µg/l) applied to the same level of cells clearly effects the shape of the degradation curves. The S-shaped curve provided by the higher concentration is characteristic of substrate inhibition and a careful evaluation of these graphs clearly provides evidence for that phenomenon. When the specific PCP degradation rates were calculated at various PCP concentrations on these graphs and plotted against PCP concentrations, the bell-shaped curve shown in Figure 33 was produced. Note that the PCP concentration at which the rates begin to decrease with increasing substrate concentration is approximately 350 µg/l. This concentration which was also the threshold concentration is approximately 350 µg/l. This concentration was also the threshold concentration for the second family of log-normally distributed data observed in the continuous flow reactors (Figures 19-22). The case for substrate inhibition by PCP is strong and its implication in reactor performance is evident.

Returning to Figure 32 it can be seen that the PCP removal curve for the flask containing 300 mg/l of cells and 2000 µg/l of PCP also was S-shaped. Consequently, the specific degradation rates were also calculated for that flask and the results are presented in Figure 34. The curve from Figure 33 is redrawn there for ease of comparison. Once again substrate inhibition is suggested by the bell-shaped curve; however, the maximum point is shifted to a higher PCP level (perhaps 800 µg/l or more) for the higher cell concentration and the maximum specific degradation rate exceeded that observed at the lower cell concentration. Chu (15) reported a similar effect in studies related to the biodegradation of PCP by Arthrobacter KC3.

The previous experiments were done using PCP as the sole substrate and led to the characteristic experimental pattern of substrate inhibition whose exact characteristics were strongly influenced by cell concentration. In the continuous flow reactor studies, PCP was supplied along with other biodegradable sustrates from the sewage, and the dog food. Therefore, an attempt was made to determine the effect of alternative organic substrates of PCP degradation and inhibition. In general, these experiments were done in a manner similar to the PCP inhibition study; the significant alteration being the addition of dog food extract to make the SCOD 100 mg/l. The specific PCP removal rates were estimated from the slopes of PCP concentration versus time plots and these rates were then plotted against the PCP concentration. A composite curve prepared from the data from the four flasks had the characteristic bell shape as shown in Figure 35. For comparison, the substrate inhibition plot obtained from the flasks without added degradable organic matter (Figure 34) is included as well as a conceptually-derived plot based on the continuous flow reactors. It should be noted that the peak PCP removal rate was very much higher with added organic matter. Nevertheless,

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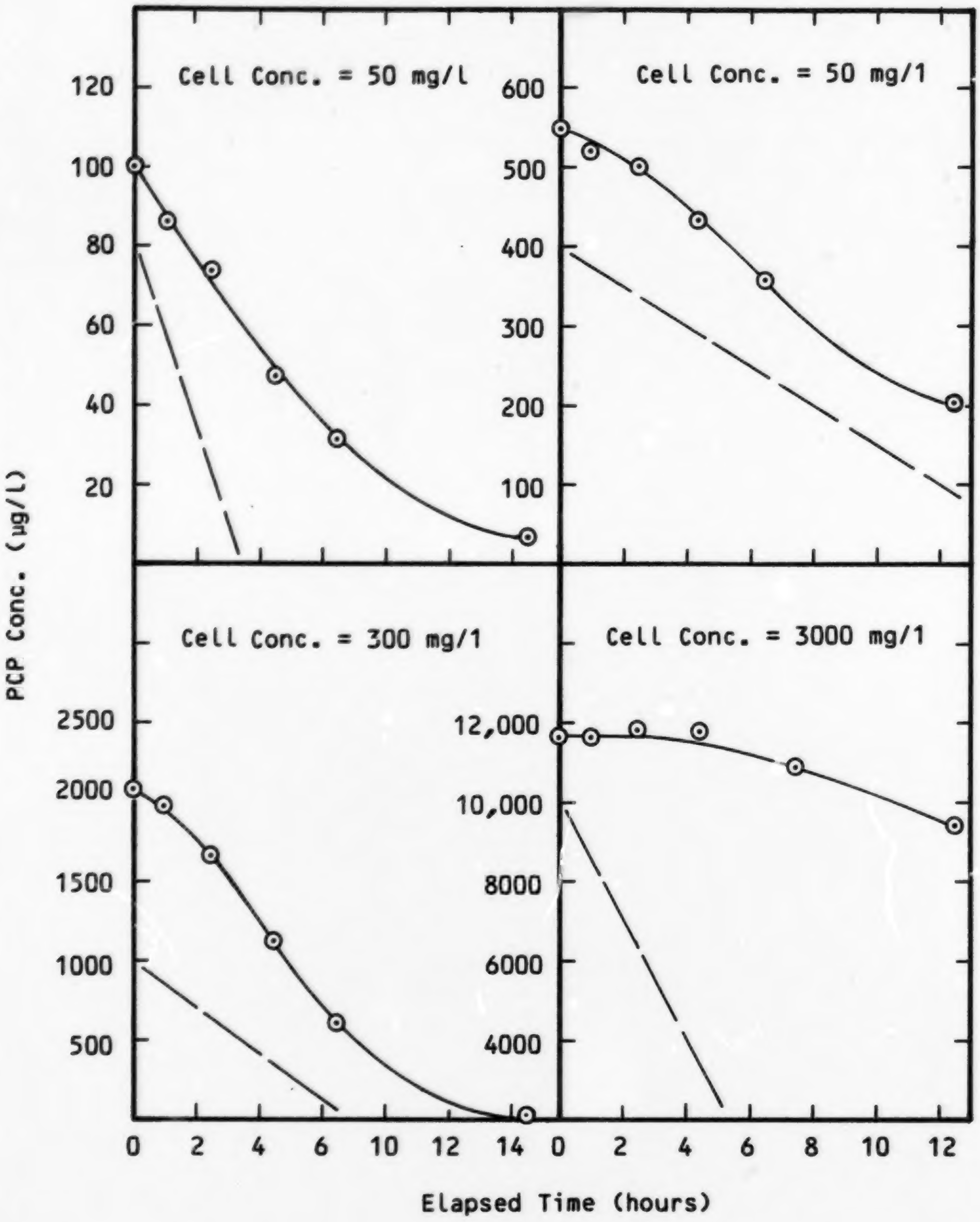

Figure 32 Removal of PCP by acclimated cells in batch experiments. PCP was the sole carbon and energy source provided. The dashed lines represent a specific removal rate of 500 µg PCP/hr·g cells.

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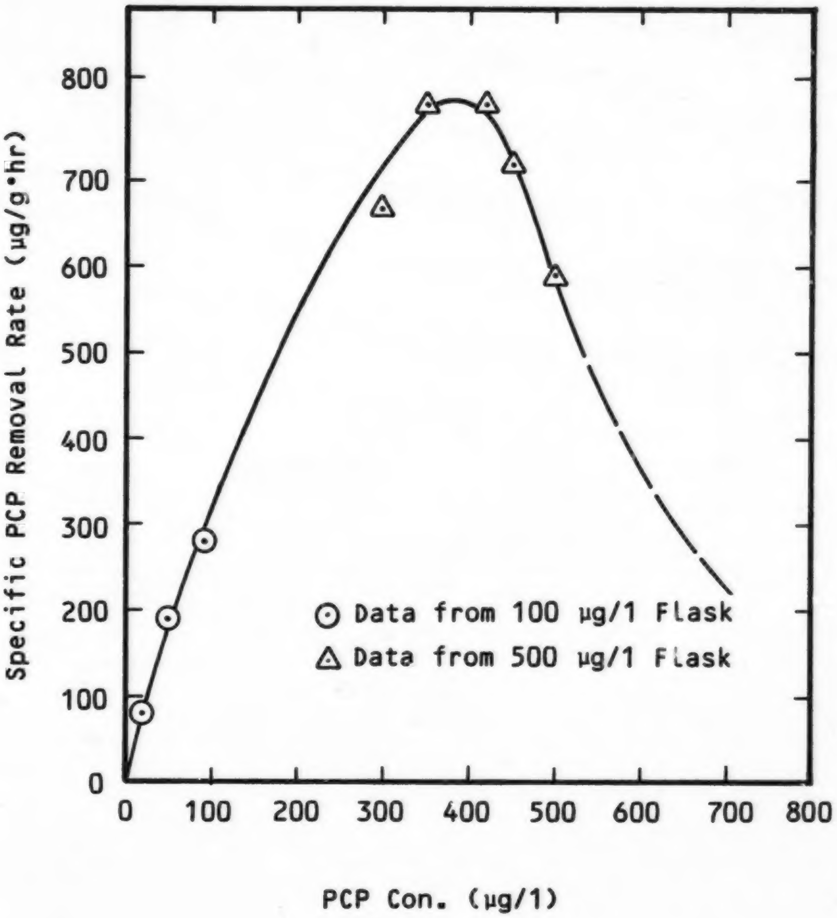

Figure 33 Effect of PCP concentration on the specific removal rate as determined from the upper two plots in Figure 32.

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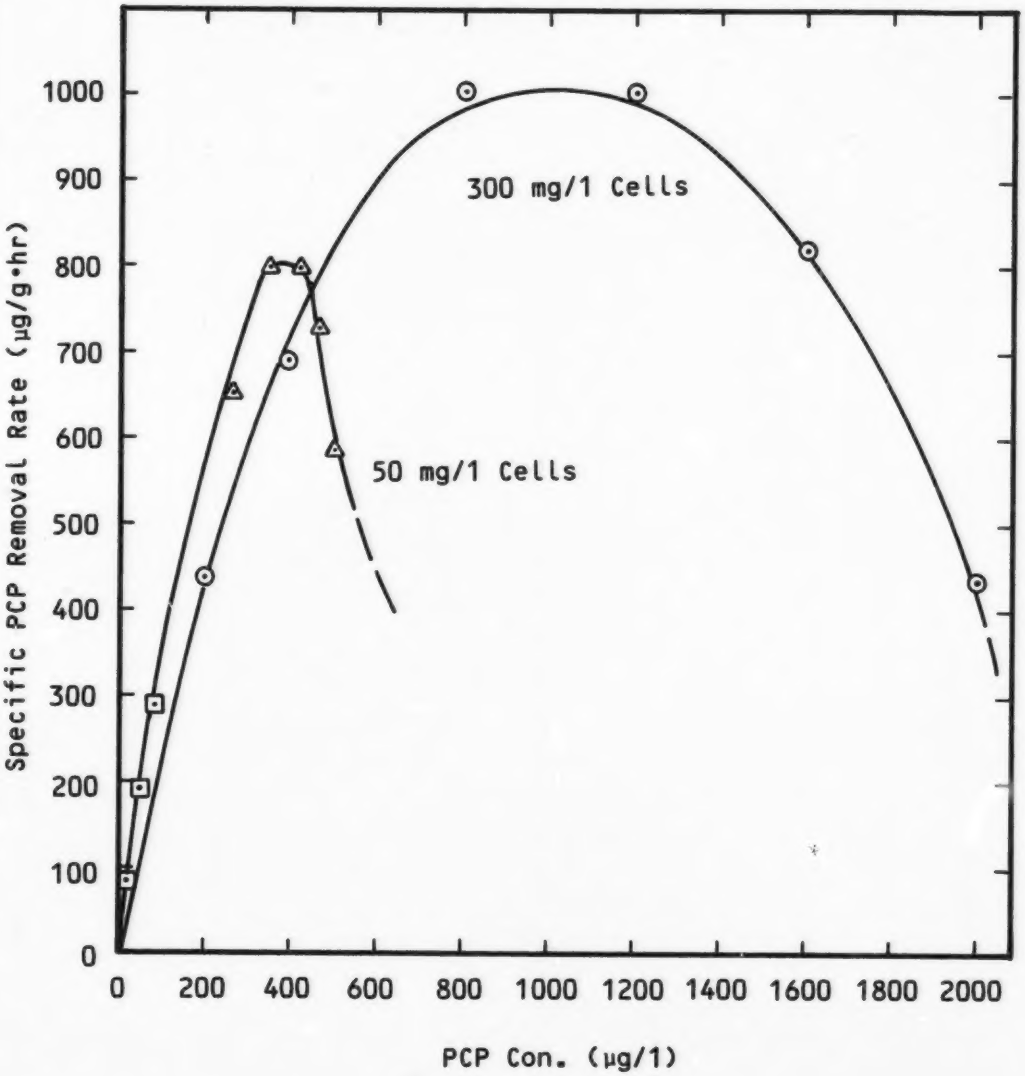

Figure 34 Effects of PCP concentration and cell concentration on the specific PCP removal rate.

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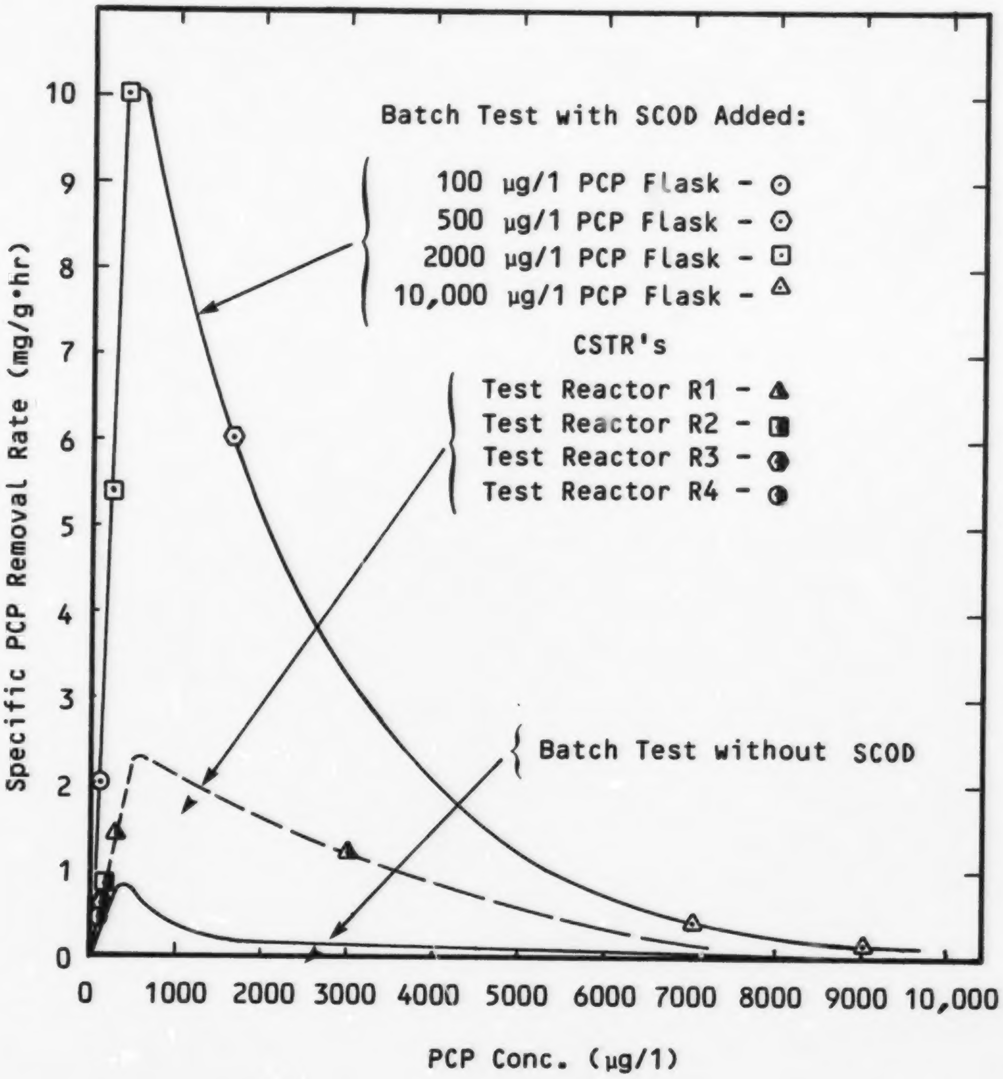

Figure 35 Effect of PCP concentration on its specific removal rate.

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the PCP concentration at which that peak occurred was not greatly influenced by the presence of other organic matter in the flasks. The peak value of the continuous flow curve (dotted line) was not experimentally determined and therefore cannot be given too much credibility. However, it is clear that the specific PCP removal rates were higher in the CSTR's, which received alternate organic substrate, than in the batch reactors which did not receive such substrate. This influence of additional organic substrates graphically demonstrates the necessity for conducting studies of this type with multicomponent media. If the studies had been done with PCP alone they would have greatly underestimated the kinetics of PCP removal likely to occur in wastewater treatment plants.

It is apparent from these studies that the bioremoval of inhibitory substances may be associated with very complex, concentration dependent interactions. Such interactions may make the use of simple substrate removal rate relationships difficult because such rates have been shown to depend upon the substrate concentration, the quality of the substrate, and the cell concentration. It is our contention, however, that kinetic analyses based upon the results from continuous flow reactors receiving a complex multicomponent media provide a more reliable approach to the prediction of the fate of such compounds in wastewater treatment systems. In any event, one can understand the reason for the greater variability of PCP concentration encountered in the reactors with short solids retention times. A reactor at a short SRT will operate at a higher average PCP concentration than a reactor at a long one. The higher the average PCP concentration, the closer the reactor is operating to the maximum point on the removal curve, and thus the greater the probability that natural variations in the influent or in system performance will push the PCP concentration across the peak into the region where the PCP removal rate decreases with increasing concentration. When such a shift occurs the PCP concentration in the reactor would continue to rise until some factor, at this point unknown, allows the biomass to adapt to the material and remove it rapidly enough to return the removal rate to the left side of the peak. A reactor with a long SRT, on the other hand, will be operating farther to the left of the peak so that the likelihood of the peak concentration being exceeded is small. Under that condition such perturbations in the character of the influent or in the performance of the system will be self-correcting and there will be less variation in the PCP concentration.

An important point to arise from this work, therefore, is that decisions concerning the design characteristics of an activated sludge plant removing an inhibitory material must consider the perturbations likely to occur in the system. Not only will a longer SRT reduce the average pollutant concentration; it will also reduce the variability associated with that concentration. In addition, the observation that the degree of inhibition exhibited by PCP is influenced by the biomass concentration introduces another consideration into activated sludge process design. For example, once the SRT has been selected, any reasonable value for the MLSS concentration or the aeration basin residence time can be chosen so long as their product is held constant. These results suggest, however, that the choice of a high MLSS concentration for a given SRT will result in a more stable treatment system because it will increase the biomass concentration in relation to the concentration of inhibitory substrate. No data are yet available which address this contention directly, so this constitutes an area that requires further research.

Evaluation of Alternative Removal Mechanisms--

As discussed earlier, once the steady-state operating points have been established for the CSTR's contributions of adsorption and volatilization to PCP removal can be determined.

Adsorption-- The contribution of adsorption to PCP removal can be determined with the isotherm in Figure 8, which was presented in the discussion of the Phase I results. Given the long hydraulic and solids retention times employed herein, it is likely that adsorption approached fairly closely to equilibrium. Thus the amount of PCP adsorbed per unit of biomass can be determined by entering Figure 8 with the mean PCP concentration and reading the adsorptive capacity from the graph. This was done for each of the test reactors and the results are given in Table 13. Multiplication of the adsorptive capacity by the mass of solids grown and removed from the reactor daily gives the mass of PCP removed per day by adsorption. These values are also shown in the table. There it can be seen that adsorption made an insignificant contribution to PCP removal, as would be anticipated from the Phase I results.

It should be recognized that for a given SRT and influent characteristic the amount of PCP removed by adsorption will be independent of the MLSS concentration employed. This is because the mass of MLSS associated with a given SRT is constant and independent of the MLSS concentration. Furthermore, so is the mass of MLSS wasted per day. Since the mass of PCP removed by adsorption is the product of the mass of MLSS wasted per day and the adsorptive capacity associated with those solids, the concentration of MLSS is irrelevant.

On the other hand, the importance of adsorption as a mechanism of PCP removal is very much dependent upon the relative concentrations of biodegradable COD and PCP in the influent. The higher the concentration of organic matter in the influent the greater the mass of MLSS associated with a given SRT, and the greater the mass which must be wasted daily. Since the adsorptive capacity depends on the average PCP concentration (which depends on the SRT) more PCP will be removed as more cells are wasted. Thus adsorption could well be a significant mechanism if the ratio of SCOD:PCP in the influent is very high. In our situation, however, adsorption was insignificant.

Volatilization--The first-order rate constant for the removal of PCP by stripping or volatilization from the stripping test apparatus was given in the discussion of the Phase I results as 0.0076 day^{-1}. An estimate of the contribution of stripping to the removal of PCP from each reactor can be obtained from:

$$-r_v V = K_v \cdot S \cdot V \tag{29}$$

where: $-r_vV$ is the mass roemoval rate of PCP by stripping (MT^{-1}).
K_v is the first order rate constant (T^{-1}).
S is the mean PCP concentration (ML^{-3}).
V is the reactor volume (L^3).

Table 13 Estimation of PCP Loss Due to Adsorption of PCP onto Biomass in Aerobic Reactors

Reactor	R1	R2	R3	R4
Nominal SRT (days)	3	7	11	15
Mean PCP Conc. (μg/l)	214	105	68	64
Adsorptive capactiy of solids at this mean PCP conc. (μg/g) (from Fig. 8)	330	200	140	120
Mass of solids grown and removed/day (mg/day)	95	75	70	68
PCP removed (μg/day)	31.4	15	9.8	8.2
% of PCP applied	0.31	0.15	0.10	0.08

*Based on 10,000 μg PCP applied to each reactor/day

Table 14 shows the results of this calculation for each reactor, based upon the assumption that the value of the rate constant is the same in all of the reactors and equal to 0.0076 day^{-1}.

The values given in Table 14 are only approximations of the PCP losses because the actual rate constant for PCP stripping will depend upon the geometry of and the air flow rate to each of the individual reactors. Therefore, more accurate estimation of the PCP stripping rates can be obtained by estimating the rate constant for each reactor individually. This can be done most easily by measuring the overall oxygen transfer coefficient (K_La) for each of the reactors and for the air stripping apparatus. The ratio of the mass transfer coefficient in a test reactor to the one in the air stripping apparatus can then be assumed to be equal to the ratio of the appropriate K_La

Table 14 Effect of Air Volatilization of PCP from the Aerobic Test Reactors

Reactor	Mean PCP Conc. μg/l	Stripping Rate μg/l·day	Reactor Volume liters	Mass Removed Per Day μg/day	Percent of PCP Fed*
R1	214	1.63	1.5	2.45	0.025
R2	105	0.80	3.5	2.80	0.028
R3	68	0.52	5.5	2.86	0.029
R4	64	0.49	7.5	3.68	0.037

*Based on an application rate of 10,000 μg/day.

values, thereby allowing calculation of the stripping mass transfer coefficient for the test reactor as discussed earlier. K_La values can be used in a similar manner to estimate the rate of stripping in a full scale activated sludge system. In this research the rate of stripping of PCP was so low that it was considered unnecessary to find the actual rate for each individual reactor. The use of such a technique would be very important, however, if a more volatile compound was being investigated.

The contibution of volatilization to the removal of PCP in actual activated sludge plants would be much lower than the estimates listed in Table 14 because the hydraulic retention times in those systems are much smaller than the values employed in this research. Such short retention times greatly reduce the time available for volatilization. Consequently, for a given SRT, the importance of stripping will decrease as the hydraulic retention time is decreased whereas the importance of adsorption will be unaffected.

Phase III Testing

As previously indicated, the demonstration of aerobic degradability in Phase II requires subsequent evaluation in Phase III of the extent of biodegradation. Two types of experiments were used to demonstrate that PCP was mineralized in the Phase II reactors. Since randomly labelled ^{14}C-PCP was available, the biologically-mediated formation of $^{14}CO_2$ was demonstrated and used as evidence for ultimate biodegradation. Alternatively, it was shown that when PCP was supplied as the sole source of carbon and energy in a continuous flow system, a new steady state was obtained which would only have been maintained for extended periods of time by de novo growth of PCP-degradaing microorganisms. This implies strongly that PCP acted as a carbon and energy source undergoing ultimate degradation via aerobic respiration.

Radiosotopic Method--

Six 125 ml Warburg flasks were charged with 25 ml of concentrated cells taken from a CSTR actively degrading PCP. The concentration of cells was 500 mg/l. ^{14}C-PCP (randomly labelled) with a nominal specific activity of 500 cpm/µg was added so that the concentration of PCP was 2000 µg/l. CO_2 was trapped in filter paper saturated with 10% hyamine hydroxide in methanol. At selected intervals 0.5 ml of 5% H_2SO_4 was added to stop metabolism and release any radioactive bicarbonate in the gas phase. The CO_2 collector strips were placed in 15 ml of scintillation fluid consisting of 0.4% 2,5-diphenyloxazole in 50% toluene:50% 2-ethoxyethanol. Radioactivity was analyzed in an ISOCAP/300 liquid scintillation counter. Appropriate corrections for background were made. Figure 36 shows that $^{14}CO_2$ was rapidly evolved for approximately 6 hours, at which time approximately 67% of the PCP carbon had been collected as CO_2. These data were interpreted to mean that PCP undergoes ultimate biodegradation and that to a large extent the disappearance of PCP in acclimated biological reactors is well correlated with its mineralization.

Non-Radioisotopic Method--

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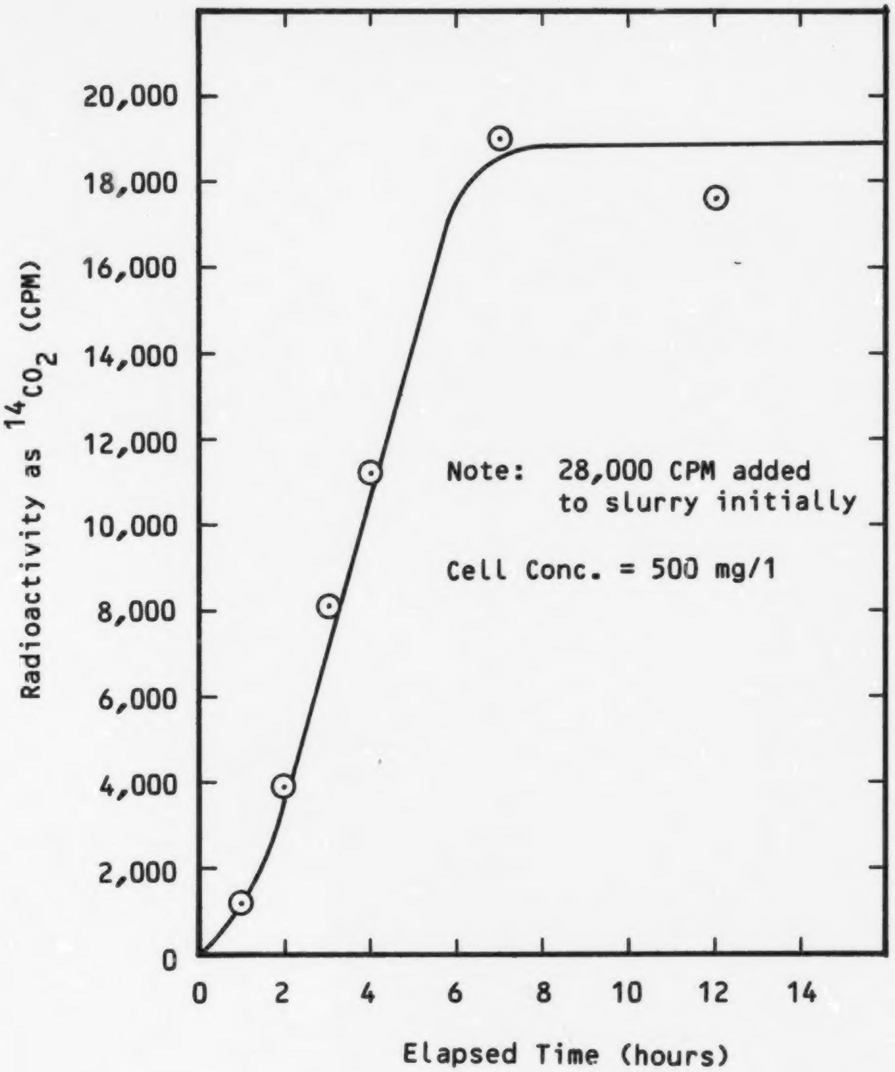

Figure 36 Release of radiolabeled CO_2 by a microbial slurry degrading randomly labeled ^{14}C–PCP.

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A 7 day SRT test reactor was dedicated to this study. The input feed was gradually depleted of all organic constituents except PCP in three stages. Initially the reactor was shifted to a feed containing filtered effluent to which 1 mg/l N as urea and 0.2 mg/l phosphate were added. The effluent was from a control reactor being fed sewage supplemented with dog food extract and it was supplied for two SRT's (14 days). For the next three SRT's (21 days), standard BOD dilution water made with well-water plus 5 mg/l yeast extract was supplied. Finally for two SRT's (14 days) BOD dilution water using deionized water plus 15% well-water and 1 mg/l N added as NH_4HCO_3 was supplied. All of these feeds contained 20 mg/l PCP as the sodium salt. When the PCP concentration in the effluent was monitored, PCP levels were shown to never exceed 600 μg/l. The data are plotted in Figure 37. Since there was ample time for cell wash out to occur, and since stripping was a negligible factor, de novo synthesis of cells must have occurred to keep the concentration of PCP well below the input level of 20 mg/l. In other words, new PCP-degrading cells must have replaced those which were washed out, demonstrating that mineralization of PCP was occurring.

Overall Evaluation of Protocol Effectiveness

In summary, it can be stated that the protocol was quite effective in allowing a determination of the fate of PCP in an aerobic wastewater treatment system. Acclimation proceeded quite smoothly with no upsets in reactor operation. The fiber-wall reactor behaved well and no operational problems were encountered. The choice of the initial PCP concentration for acclimation and the schedule for increasing the fraction of PCP degrading organisms in the biomass were both satisfactory, but in retrospect one must ask whether that was simply fortuitous. As will be seen in the next section, difficulties were encountered in anaerobic acclimation so that it was necessary to incorporate a toxicity screening protocol as a part of Phase I testing. Such a protocol, which could be run with unacclimated activated sludge growing on fortified sewage in a Warburg apparatus, would help define the pollutant concentrations likely to cause inhibition of the biomass thereby providing a rational basis for choosing the initial acclimation concentration and for establishing the schedule for increasing the pollutant concentration in the feed. Such an addition to the protocol would require a minimum expenditure of effort and could actually reduce the total effort required by either speeding up the enrichment process or by avoiding the necessity for restarts caused by toxicity.

Regardless of whether a screening assay is employed, the step-up schedule employed should be flexible and should reflect the demonstrated capability of the biomass rather than some arbitrarily selected values. To this end it is important that the specific assay for the test compound be available during acclimation so that it can be used to determine when the next step-up is being made successfully. Finally, it must be emphasized that Phase I testing cannot be rushed. It must proceed at the rate determined by the biomass and that rate can only be ascertained by testing.

Phase II testing was successful in establishing a functional relationship between the specific growth rate of the culture (as reflected by the SRT) and the level of PCP remaining in solution. Thus, it should be possible

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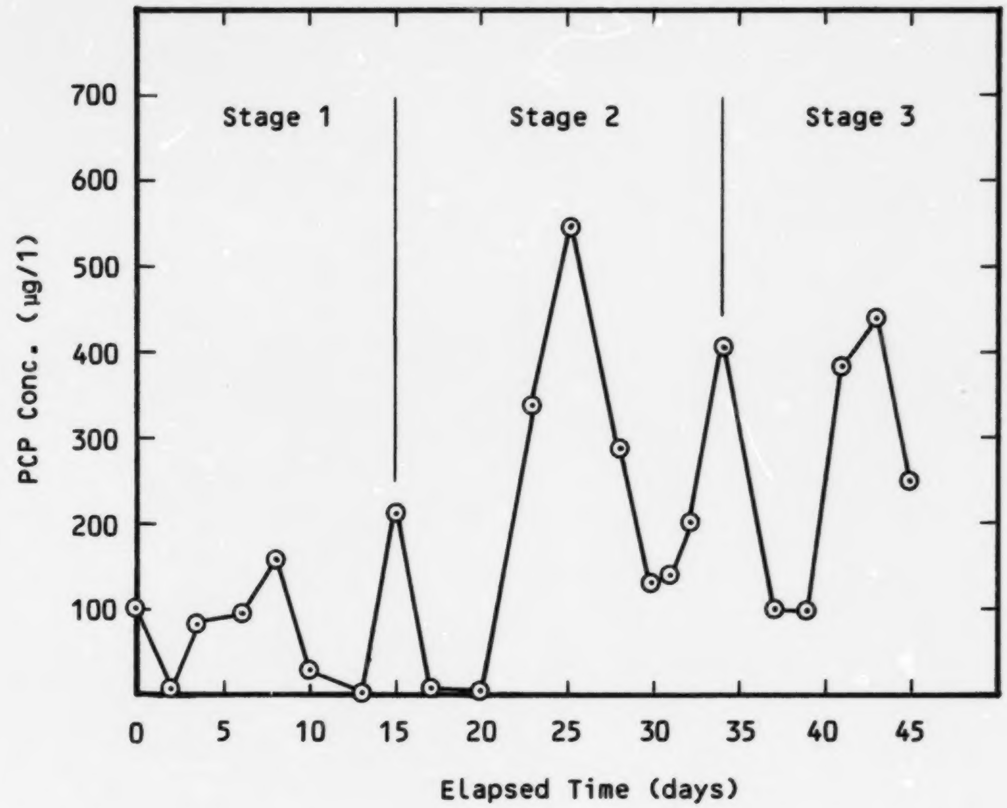

Figure 37 Concentration of PCP in the effluent from a CSTR with an SRT of 7 days. Each stage represents a period within which PCP contributed more and more of the influent carbon(see text). During Stage 3 PCP was the sole carbon and energy source provided.

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to predict the level of PCP remaining at any SRT within those limits for a wastewater that is "Typically domestic". Furthermore, it was possible to use the data on sorption and volatilization obtained in Phase I testing to show that those mechanisms were minor for PCP. More severe tests of the applicability of those procedures will be obtained in later investigations when compounds that are more volatile or more sorptive are encountered. It may be necessary at that time to adopt additional procedures, such as the entrapment of volatilized pollutant, in order to check the accuracy of the procedures employed herein. The low contribution of volatilization and sorption to the removal of PCP made such checking impossible in the present study.

The necessity for long-term testing in Phase II was clearly demonstrated in this work as was the necessity for dealing with the data on a statistical basis. Although the kinetic approach was capable of predicting the average removal of PCP associated with any given SRT, the long-term data demonstrated clearly that the stability of the reactors was closely associated with the SRT values. Secondary studies demonstrated the substrate-inhibition kinetics associated with PCP degradation and suggested that those kinetics were associated with the instability observed at short SRT values. Since typical steady-state CSTR reactors cannot be utilized to develop the entire kinetic curve for an inhibitory substrate and since the inhibitory function appears to be highly complex (involving not only the concentration of the test compound but the concentrations of other substrates and the biomass as well) it does not appear feasible at this time to attempt to determine the entire inhibitory kinetic function as a part of routine testing of biodegradability. However, relatively straight-forward experiments such as those run herein can provide considerable insight into where problems might be encountered so that those operational conditions can be avoided. This work has demonstrated, however, the necessity for fundamental studies on the dynamic kinetics of the degradation of inhibitory substrates in the presence of other carbon sources.

One question posed at the start of this work was whether some commonly occurring "internal standard" could be used to estimate the removal of a priority pollutant without having to conduct a specific analysis for the pollutant. Two surrogate materials were considered: SCOD and MBAS. While the long-term average concentrations of each were highly correlated with the PCP concentration (because of the effect of SRT on each), there was no correlation with daily PCP values. This was probably due to the inhibitory effect of PCP upon its own removal. Regardless of the cause, the results clearly demonstrated that a surrogate test cannot be used to indicate the fate of a priority pollutant.

The experiments in Phase II also allowed an evaluation of the effects of the test compound upon the normal performance of aerobic biological treatment. Again, such an evaluation must be made on a statistical basis to be sure that any observed effects are significant. One factor which the protocol does not evaluate directly is the effect on sludge settleability. If desired, an estimate of that effect could be obtained by running a column settling test on the cells removed daily with the effluent.

The radiolabeled substrate experiments in Phase III provided very good

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circumstantial evidence that PCP was undergoing relatively complete biodegradation. Similarly good circumstantial evidence was provided by the experiments in which a CSTR with an SRT of seven days was operated with PCP as the sole carbon and energy source. Because of the general lack of availability of labeled priority pollutants the second alternative will probably be used in routine screening. It is important to recognize, however, that while a positive result indicates relatively complete biodegradation of the test compound a negative result means only that the test compound cannot serve as a sole carbon and energy source. It does not show that the test compound cannot be completely degraded in the presence of other carbon and energy sources. The ultimate fate of the test compound in the presence of other carbon and energy sources can be ascertained with radiolabeled experiments and perhaps a logical sequence of testing would be to employ radiolabeled experiments only to those compounds which fail to support growth as the sole carbon and energy source.

In conclusion, the protocol was generally successful and was relatively easy to conduct. It should be recongized, however, that the low volatility and sorbability associated with PCP made it impossible to strenuously test those aspects of the protocol. Consequently, there is a strong likelihood that further modifications to the protocol may be necessary as more experience is gained with compounds which are subject to more volatilization or sorption. In the meantime, the protocol as proposed and ammended represents a reasonable approach to the determination of the fate of compounds with low volatility and sorbability.

ANAEROBIC TESTING

The objectives of this research were clearly stated earlier in this report. A number of questions related to those objectives were generated and experiments were designed to answer those questions. In many instances the questions were the same as those posed for the aerobic testing procedures.

1). Using the culture system described as the Phase I acclimation reactor and the acclimation schedule outlined, can an anaerobic biomass be acclimated to provide significant reduction in the PCP concentration?

2). Based on operational experience, what modifications, if any, are needed to refine the acclimation phase?

3). What are the effects of SRT on "steady-state" levels of PCP in Phase II digesters?

4). How can the data generated be used to predict the removal of PCP in anaerobic digesters?

5). What, if any, effect does PCP have on "normal" performance of Phase II reactors?

6). To what extent do competing nonbiological elimination mechanisms effect observed decreases in PCP concentration?

Phase I Testing - First Trial

After a period of operation to build up biological solids with raw primary sewage sludge as the feed, the stepwise addition of PCP was begun at 1.5 mg/l for 5 days at which time it was decreased to 1.0 mg/l for 5 days and then increased by 50% every 5 days thereafter. By the end of 60 days, the input concentration of PCP was 20 mg/l. Table 15 shows the estimated concentration of PCP in the reactors at each 3 day interval provided that no degradation or sorption of PCP was encountered.

Table 15. Calculated Reactor Concentration of PCP if No PCP Removal

Time (days)	Estimated PCP Conc. in reactor (mg/l)
0	0
5	0.23
10	0.35
15	0.52
20	0.78
25	1.17
30	1.75
35	2.62
40	3.93
45	5.90
50	8.85
55	13.25
60	19.92

Note that this scheme kept the concentration of PCP below 2.0 mg/l for at least 30 days. The estimated hydraulic retention time (HRT) was 32 days and the solids retention time (SRT) was 73 days.

The operation of triplicate PCP acclimation reactors (#4, 5, 6) relative to triplicate control reactors without PCP (#1, 2, 3) was compared by plotting the ratio of the gas volume of each PCP reactor to the mean gas volume of the controls. Throughout the test period, the gas outputs of the control reactors were fairly constant at about 1.4 liters/day. These data are shown in Figures 38-40 by the solid line plots. The open bars in each graph refer to the calculated PCP concentration as given in Table 15 and the solid bars indicated the measured soluble concentration of PCP. Note that the units of the scales of PCP concentration are markedly different. The concentrations of total volatile acids in the digesters are plotted in Figure 41.

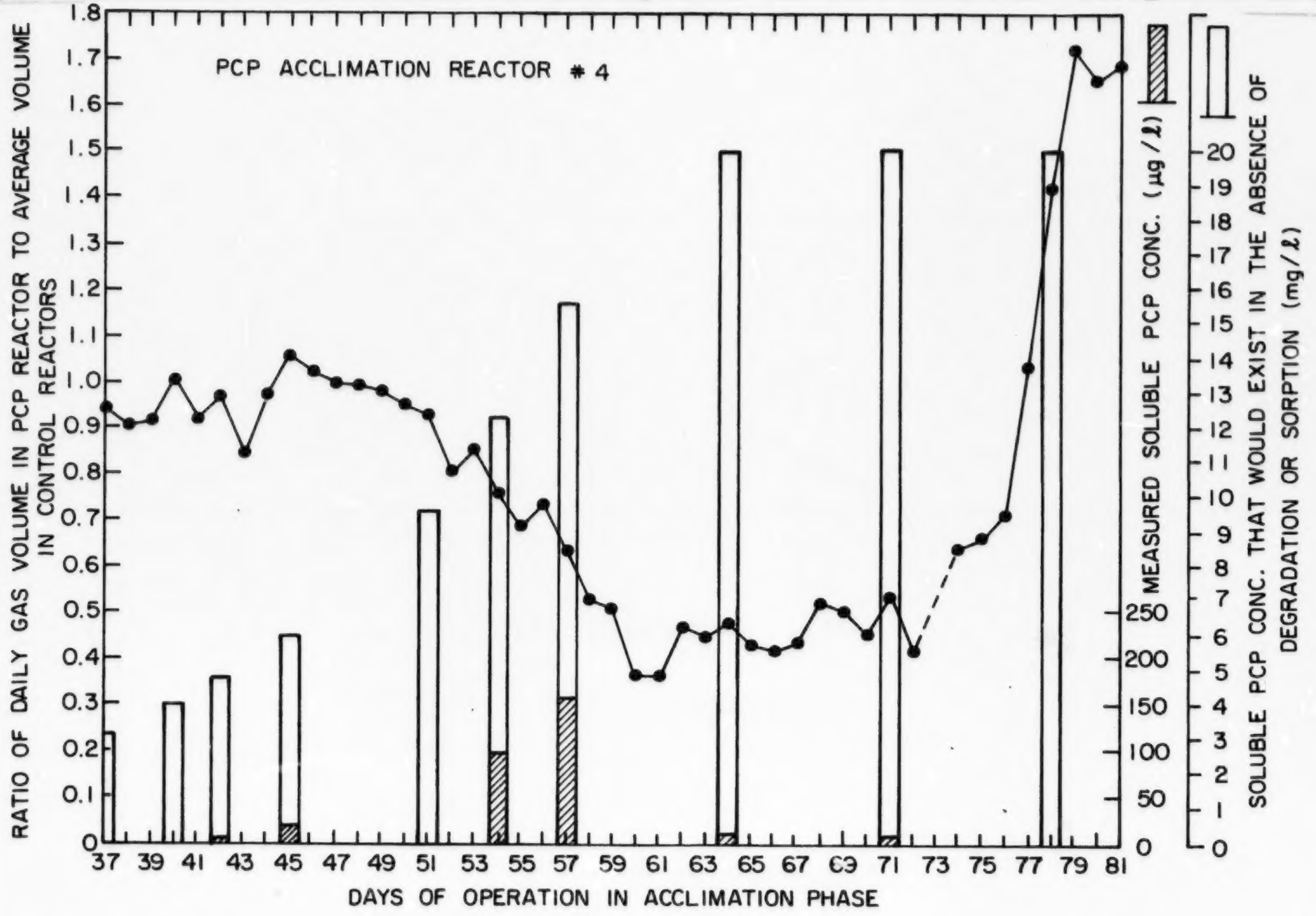

Figure 38 Gas production rate and PCP concentration in acclimation reactor 4 during Phase I.

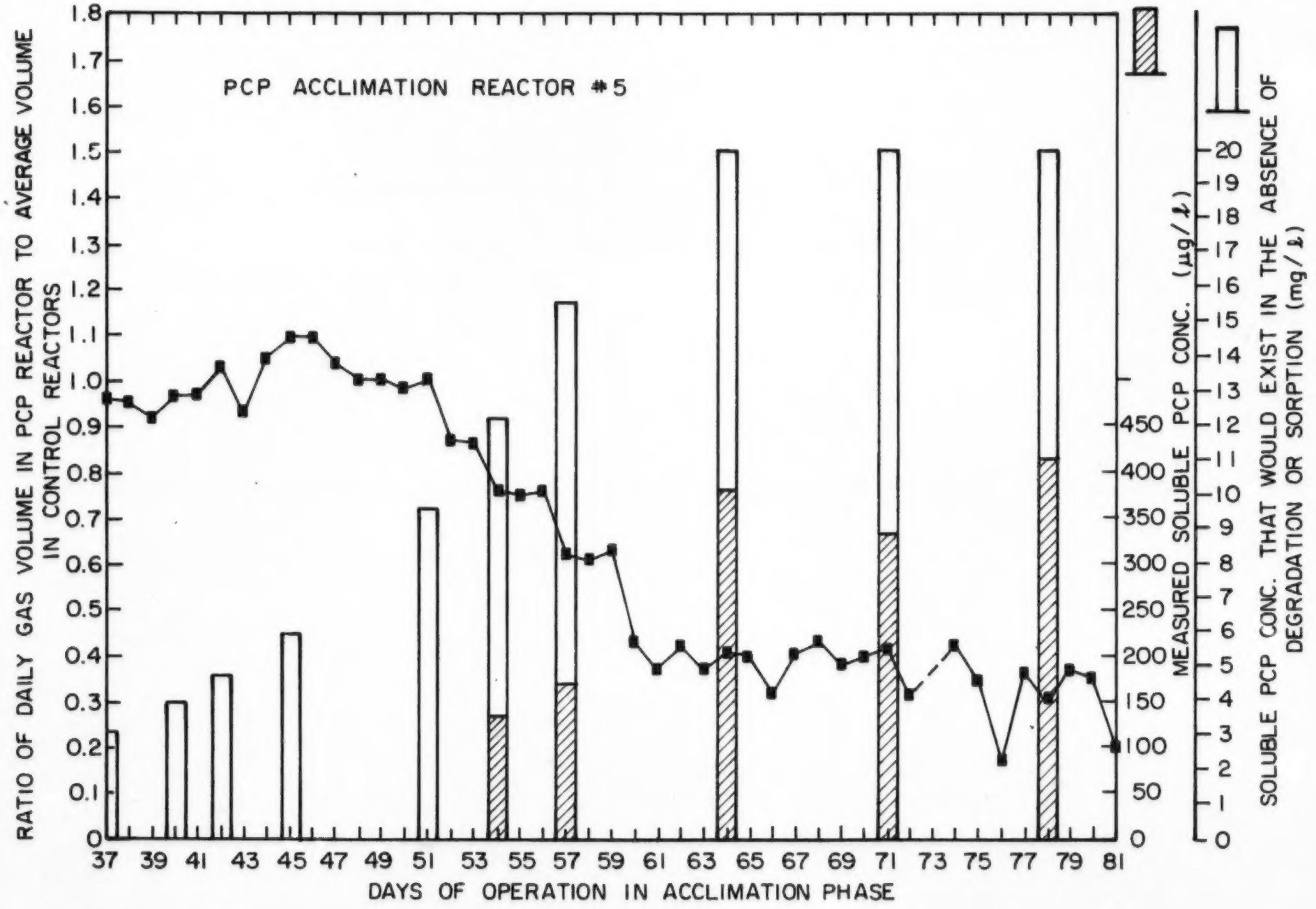

Figure 39 Gas production rate and PCP concentration in acclimation reactor 5 during Phase I.

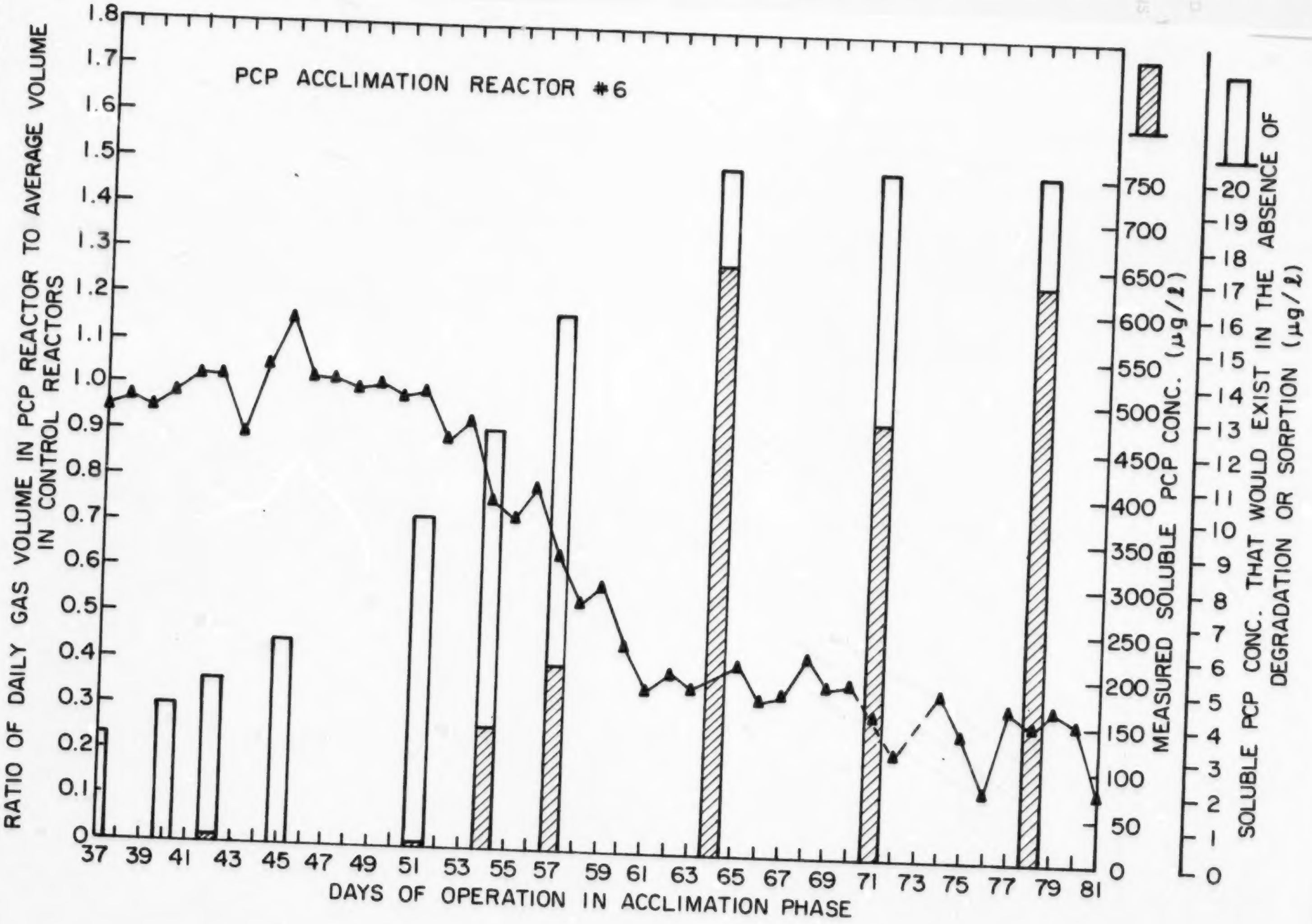

Figure 40 Gas production rate and PCP concentration in acclimation reactor 6 during Phase I.

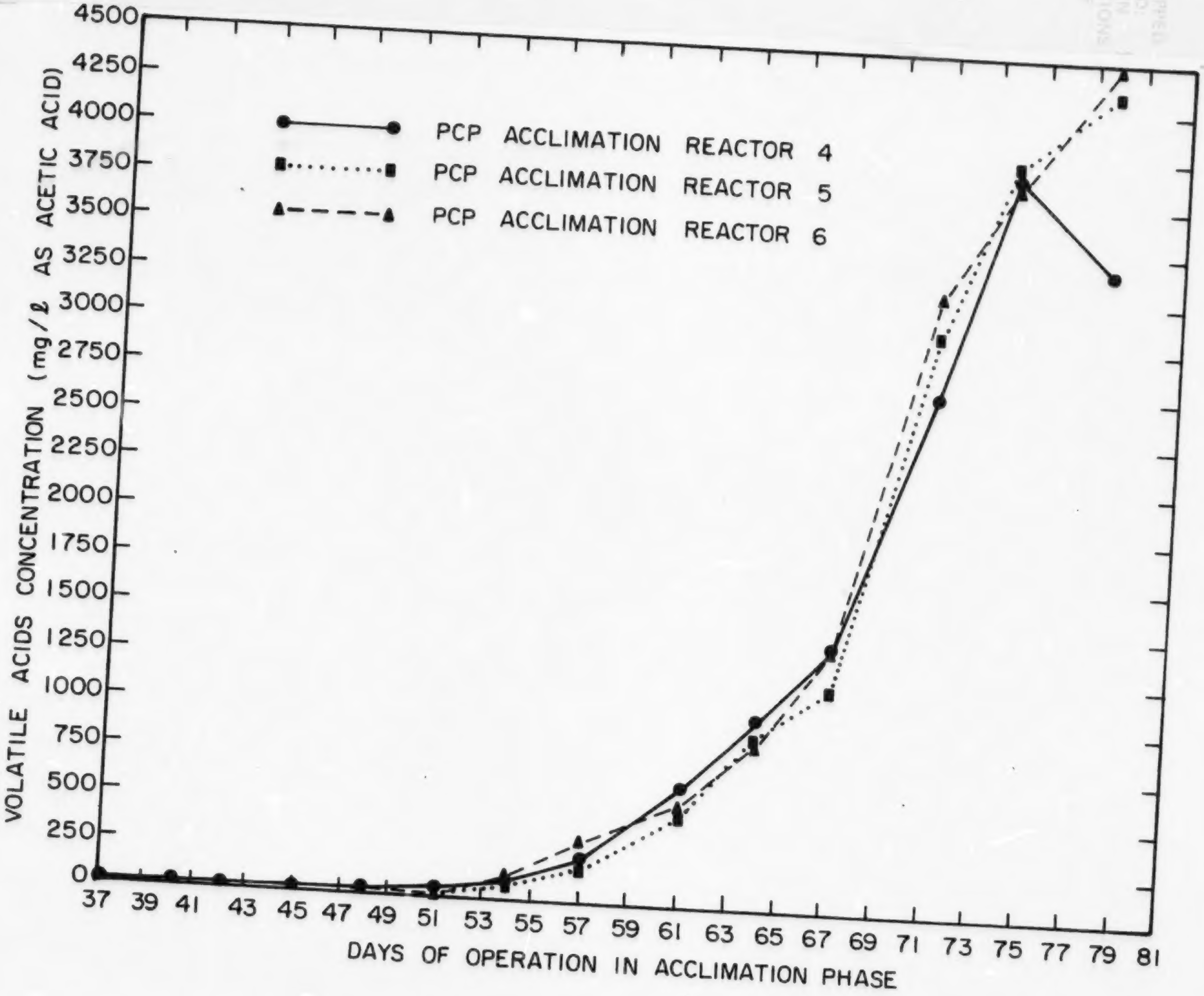

Figure 41 Volatile acids concentration in acclimation reactors 4, 5, and 6 during Phase I.

The performance of the test reactors was very similar to the controls for the first fifty days, with steady gas production, steady pH, constant alkalinity and low volatile acids. During that time little or no PCP could be detected in spite of the fact that the concentration in the reactors would have been 8.85 mg/l had there been no removal. After day 50, the rate of application of PCP increased materially due to the feeding protocol and evidently outpaced the ability of the biomass to remove it because performance of the PCP reactors began to deteriorate. As can be seen in the abovementioned figures, gas production decreased and volatile acids increased markedly, indicating inhibition of the methane-forming bacteria. Accompanying these changes was an increase in the soluble PCP concentration. After day 60, the feed PCP was maintained at 20 mg/l and performance appeared to stabilize, but at a reduced rate of gas production. The volatile acid concentration, on the other hand, continued to rise, indicating that the rate at which the methanogens were working was insufficient to keep up with the rate of acid production. Although the protocol called for initiation of Phase II testing after 60 days, the reduced methanogenic activity suggested that a biomass transfer at this point would be deleterious, therefore, the acclimation was extended. Eventually (around day 68) it became necessary to add bicarbonate alkalinity to the system to maintain the pH at neutrality.

After day 60 the performance of reactor 4 began to deviate from the other two. The main effect noted at first was a reduction in the soluble PCP concentration, although soluble PCP in reactors 5 and 6 continued to increase. Then after day 72, the gas production rate in reactor 4 began to increase, eventually exceeding that of the controls. The accumulated volatile acids decreased as gas production increased. Gas production continued to rise. Thus, it appeared that reactor 4 had been able to reduce PCP concentration below inhibitory levels, thereby allowing it to recover. Examination of the data indicate that soluble concentrations of PCP in excess of 150 μg/l seem to be associated with considerable deterioration of the methene-forming process. Why reactors 5 and 6 did not overcome PCP inhibition is not clear. Perhaps random differences in performance were enough to keep the concentration of PCP in reactor 4 below the threshold of strong inhibition, thereby allowing it to recover.

Another interesting question to arise from these data is why the rate of gas production in reactor 4 did not rise until day 74 in spite of the fact that essentially no PCP was found on day 64? One answer could be that the inhibition was being caused by a degradation product of PCP and that it was still in the reactor until after day 74. In support of this, the gas chromatograms showed the existence of peaks which were not characteristic of PCP or of the control effluent, suggesting that degradation products were indeed being released to the medium.

To summarize, the first anaerobic acclimation study of PCP degradation gave some surprising results. Specifically, the fact that the soluble PCP levels in the test reactors did not exceed 650 μg/l despite an input concentration of 20 mg/l indicates that one or more removal mechanisms were at work. Furthermore, the existence of several new peaks on the chromatograms suggests that possible degradation products were being formed, therefore implying that some biological activity was in part responsible for removal. Furthermore, this study pointed out that the arbitrary choice of 20 mg/l as an input concentration of a bio-inhibitory priority pollutant might lead to a low activity anaerobic biomass which would be unsuitable for starting Phase II reactors. In fact, the reactors were very slow in returning to "normal" operation even after a long period of operation without input PCP. It is quite likely that PCP or PCP intermediates were adsorbed to the sludge and continued to partially inhibit activity even though the soluble PCP concentration was very low. This problem delayed initiation of Phase II testing and clearly demonstrated the need for a rapid screening procedure to signal as early as possible any untoward effects a priority pollutant might have on the digester biomass. A meaningful assay would permit more judicious selection of a nontoxic maximum dosing level for the acclimation reactors, and allow development of unihibited sludge for Phase II. The short term anaerobic toxicity test described by Owen, et al., (12) appeared ideal for that purpose and thus Phase I was repeated with it as the first step.

Phase I Testing - Second Trial

Anaerobic Toxicity Assay--

The toxicity bioassay is described in the Methods section. It was used to determine the relationship between PCP concentration and gas production in a digesting biomass not previously exposed to PCP. Since methanogenesis is a critical final step in the complete digestion process, toxicity to this microbial population can result in decreased performance and progressive failure of the system. This test was applied to PCP and Figure 42 shows the cumulative gas production plotted against time for each PCP concentration tested. The gas production rates are included in the legend. The untreated controls gave a gas production rate of approximately 16.0 ml/day after an initial 40 hour adjustment period. PCP-treated cultures at 20 and 60 μg/l PCP showed no signs of inhibition; in fact, gas production was slightly elevated. At 200 μg/l the gas production rate fell off to 14.8 ml/day and substantially decreased with further increases in the concentration of PCP. A concentration: activity relationship can be drawn for these data (see Figure 43) to locate the inhibitory threshold concentration. It appears that inhibition begins at a concentration in excess of 140 μg/l and is essentially complete at 2 mg/l.

These data relate well to the fact that in the first acclimation study, the gas production decreased when the soluble PCP concentration in the reactor exceeded 150 μg/l. Furthermore, the data suggest that the PCP dose in an acclimation system should not exceed a value giving an equilibrium PCP concentration of 150 μg/l. This method seems very useful for establishing the dosing regime during acclimation and consequently it has been adopted as part of the standard protocol for anaerobic testing of priority pollutants.

Acclimation--

A series of three acclimation reactors was again started as previously described. The PCP feeding schedule was not altered; however, on the basis

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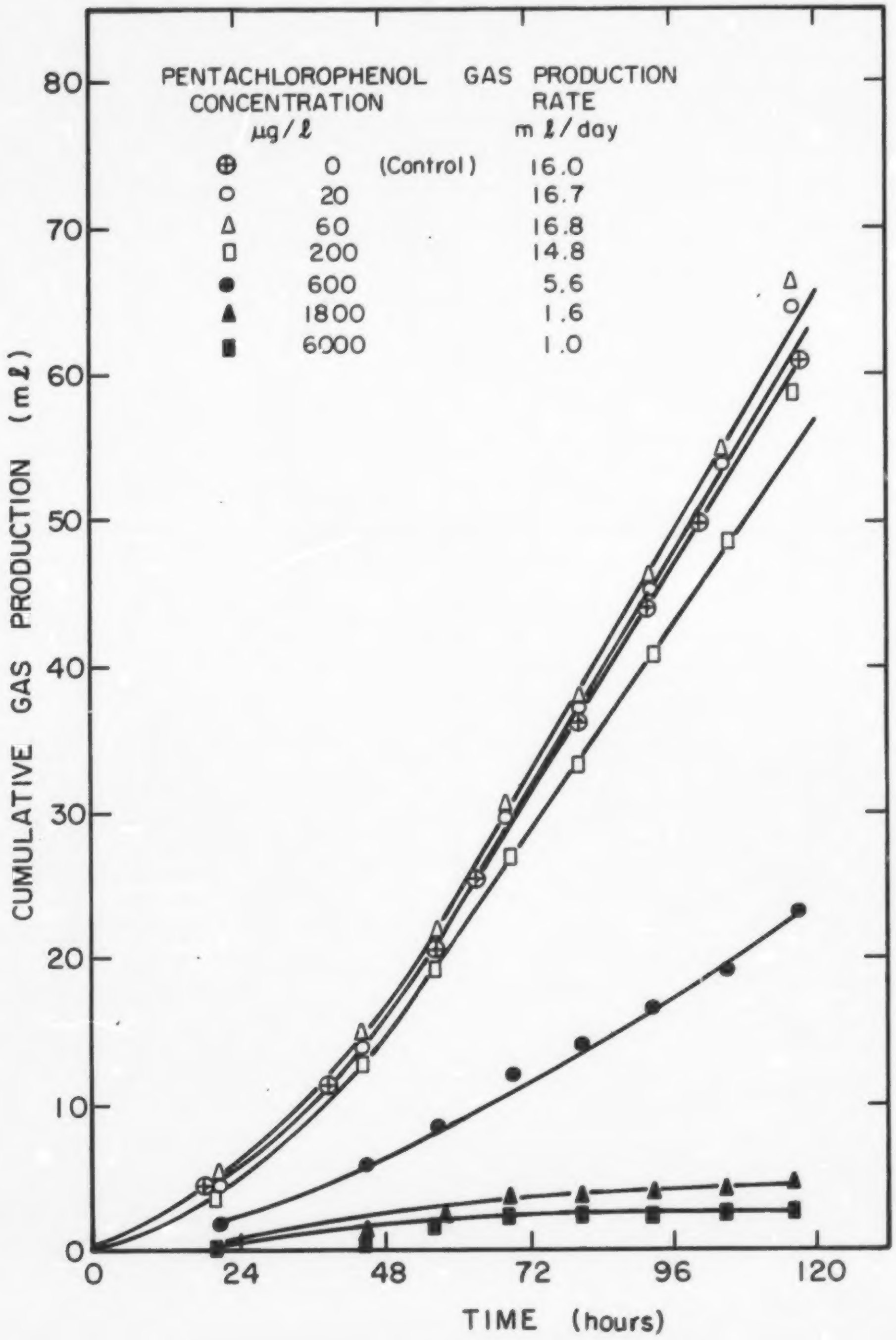

Figure 42 Results of the anaerobic toxicity assay.

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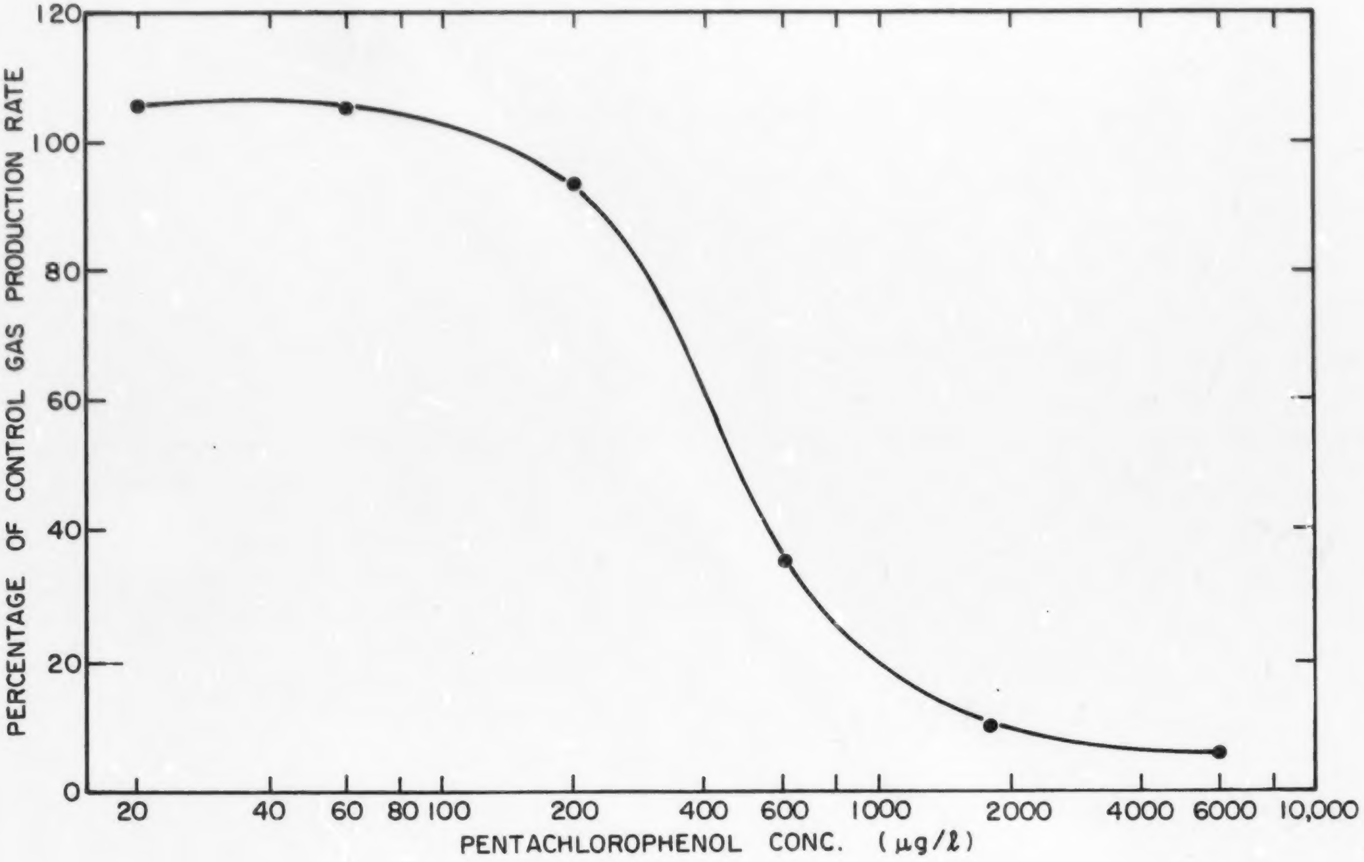

Figure 43 Effect of PCP concentration on the rate of methane production by anaerobic biomass without prior exposure to PCP.

of the anaerobic toxicity study it was recognized that if the soluble level of PCP should exceed approximately 200 μg/l for any length of time, the possibility of significant inhibition (10%) could occur. Consequently, the increase in the level of PCP in the feed was terminated when the concentration reached 7.6 mg/l and the acclimation phase was continued at the level, which on the average kept soluble PCP fairly low.

A graphical record of the performance of the acclimation reactors is shown in Figures 44-46. The data for each reactor are presented individually so that the peculiar operating characteristics of the reactors can be examined and compared: Note, however, that reactors 4, 5 and 6 were all operated identically, receiving the same PCP concentrations and the same raw sludge feed which was taken from a single well-mixed sludge stock and used during the entire acclimation period.

The soluble PCP levels were observed to be quite variable, even after months of constant influent conditions. Values ranged from below detectable limits (less than 10 μg/l) to peaks of nearly 500 μg/l. The most remarkable feature of this variability is that the PCP concentrations appear to follow a pattern of cycling between high and low concentration and that this pattern is closely replicated in each of the three reactors. Visual observation shows the period associated with the high PCP concentration was around 35 days. A probability distribution of PCP concentrations measured in reactor number 6 for the final three months of operation shows that roughly one third of the time PCP levels can be expected to be below 25 μg/l in the reactor effluent (see Figure 47). The rest of the time, concentration fell into a normal distribution ranging from 25 to 500 μg/l.

Several possible explanations of the cycling phenomenon may be advanced which are compatable with these observations. Briefly, two theories which should be considered are:

1). Minimum induction concentration of PCP degradation.

2). Inhibition by PCP or degradation products.

The fact that the period of PCP cycling is short (35 days) compared to the SRT (73 days), suggests a "physiological model" might explain this phenomenon. Perhaps a minimum level of PCP in the 200-300 μg/l range is required to adequately induce enzymes responsible for PCP breakdown. Following induction, rapid removal of PCP would occur and low effluent concentrations of PCP would be maintained. If these concentrations were beneath the induction level, however, enzyme synthesis would cease. Then dilution or degradation of the PCP-enzymes would reduce their activity enough to allow concentrations of PCP to rise, until the optimal inducer concentration was once again reached and the cycle begun again. It should be noted that only minor changes in the PCP removal rate are associated with the accumulation and reduction of PCP in the reactors. For example, the calculated steady-state PCP removal rate (i.e., when the concentration is low) was 233 μg/l•day whereas the rate during the period of increasing PCP concentration was 225 μg/l•day. This suggests that apparently minor changes in the removal rate can have a large effect upon the concentration in the

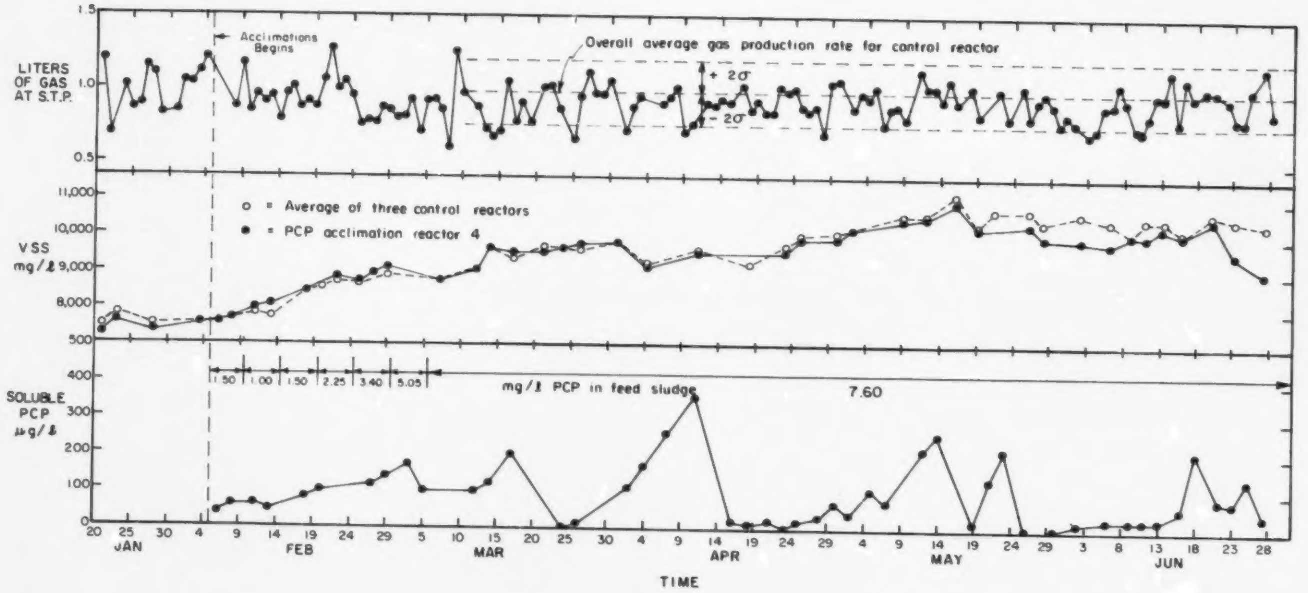

Figure 44 Gas production and concentrations of volatile suspended solids and PCP in acclimation reactor 4 during the repeat of Phase I.

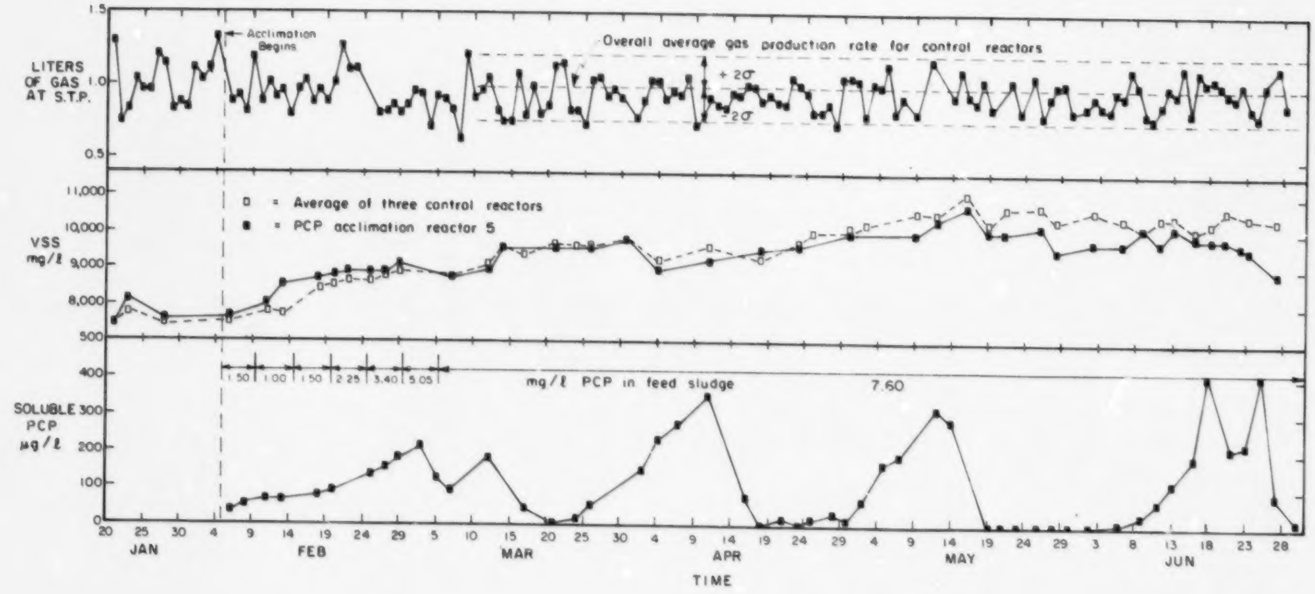

Figure 45 Gas production and concentrations of volatile suspended solids and PCP in acclimation reactor 5 during the repeat of Phase I.

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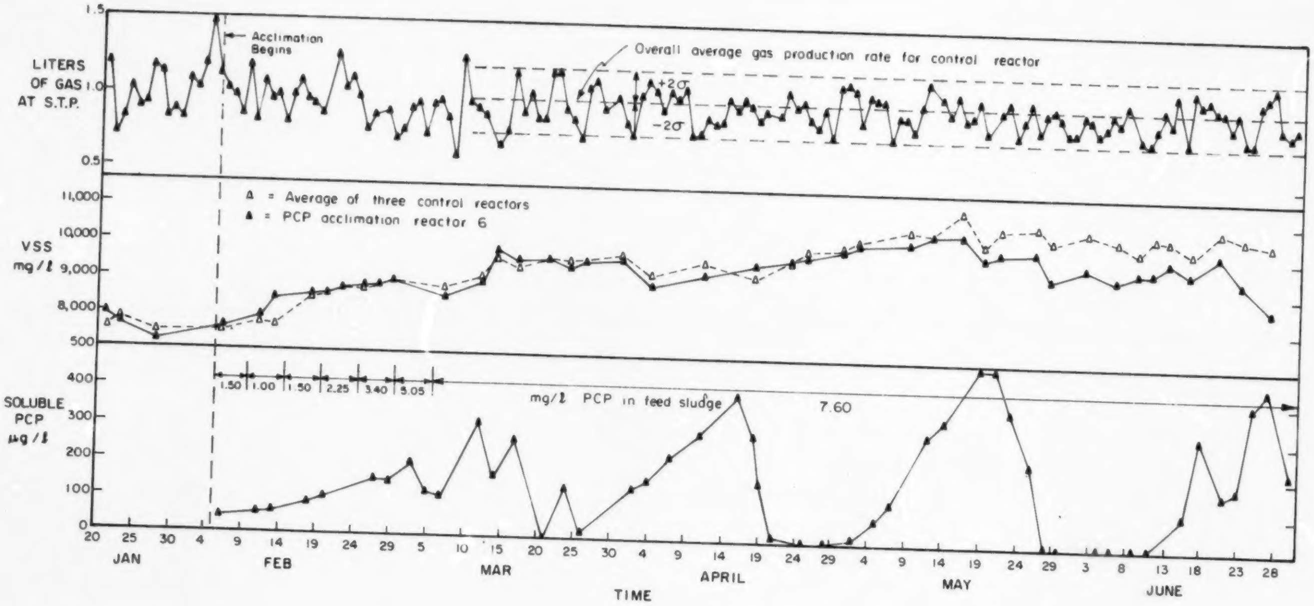

Figure 46 Gas production and concentrations of volatile suspended solids and PCP in acclimation reactor 6 during the repeat of Phase I.

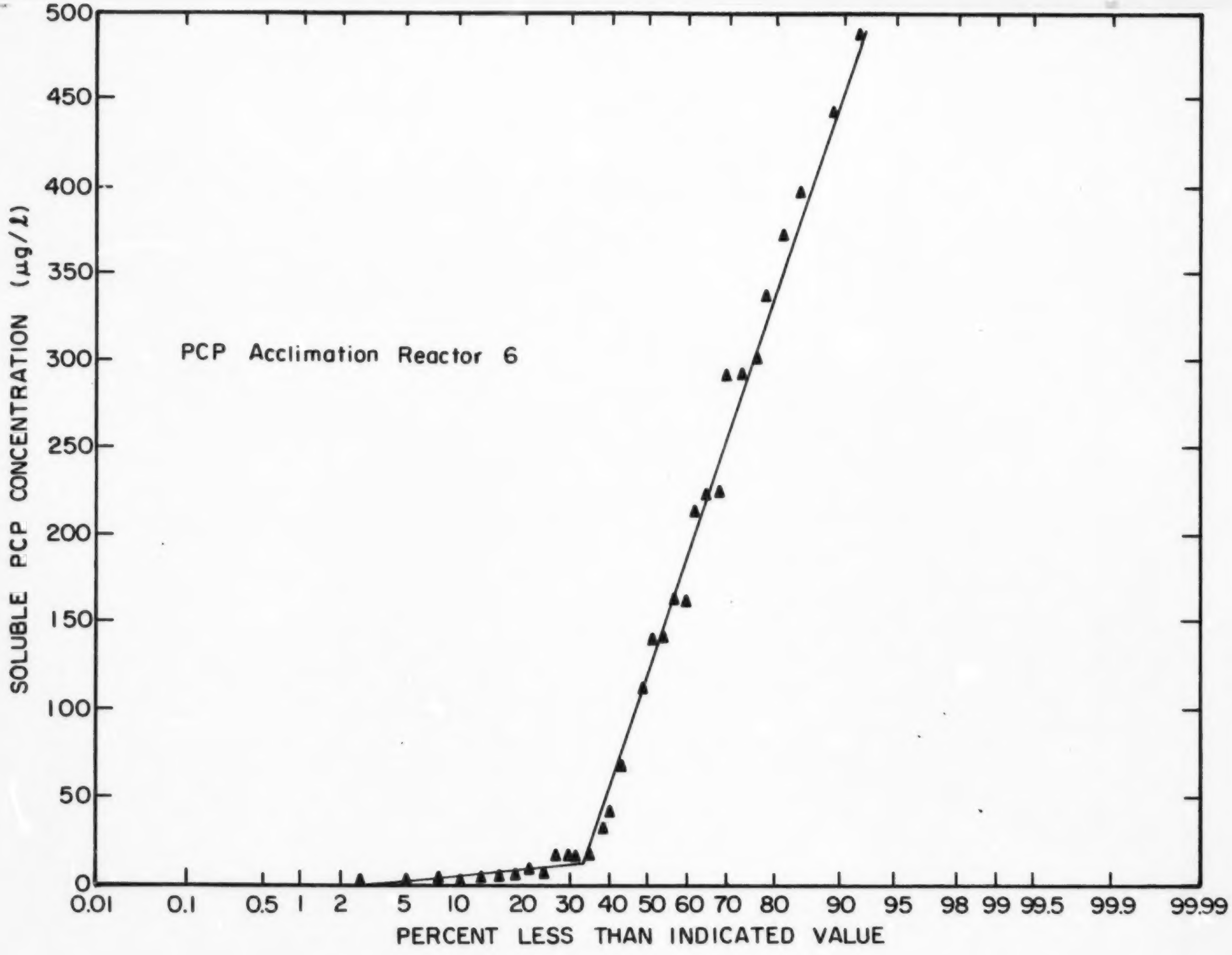

Figure 47 Probability plot for PCP concentration in acclimation reactor 6 during three months of operation (April – June).

reactor. Similarly, during periods of decreasing PCP concentration the PCP removal rate fell between 300 and 320 µg/l·day. Thus it can be seen that only minor changes in enzyme activity could account for the observed cycles in PCP concentrations.

The alternative explanation is that a breakdown product of PCP is inhibitory to the primary degradation of PCP. Such a situation has been observed in other systems and is compatable with other observations we have made. Basically, as PCP is degraded, and its concentration is reduced to low levels, some unidentified breakdown product might accumulate in the system to a level which is sufficient to reduce enzymatic activity on PCP, resulting in the accumulation of PCP. Then, when the level of this inhibitory substance is reduced, through further degradation or dilution, PCP removal may occur at a higher rate until the inhibitor concentration again builds up. Identification of the specific inhibitor(s) which might be responsible for the observed cycling of PCP is beyond the scope of this project. Nevertheless, as mentioned earlier extraneous peaks have been observed on the chromatograms from the PCP test reactors and identification of the compounds associated with them would be very revealing. Without such studies as well as others it is impossible to determine conclusively the cause of the oscillations. However, additional evidence in Phase II testing suggests that the second cause is most likely.

The operating parameters normally used to assess the performance of anaerobic digesters have not shown any significant effects of PCP on these systems. pH and alkalinity values were nearly identical between PCP and control reactors, and volatile acids, although highly variable, were only slightly higher in the PCP reactors. Typical values were pH = 7.1; Alkalinity titrated to pH 4.0 = 2550 mg/l as $CaCO_3$; volatile acids = 400 to 1200 mg/l as acetic acid. The methane fraction of the off-gas was the same for all reactors, with about 63 percent CH_4 and 33 percent CO_2.

Gas production in the test reactors was slightly lower than in the controls, but the difference does not seem significant. Since the reactors were going through a transition period the question arose as to whether the mean values could be compared. That could only be done if the time-dependent changes in the gas production rates were negligible. To find out how important time dependent changes were, the gas production data collected after a steady influent PCP level of 7.6 mg/l had been reached were subjected to a linear regression analysis with time as the independent variable. The slopes and correlation coefficient for each of the reactors are shown in Table 16 along with the mean values, and standard deviations. Examination of that table shows that time had a negligible effect upon the gas production rates and that the majority of the variation was due to other causes. Thus the mean values could be compared. Comparison of the means and standard deviations shows that in all cases they overlap and that the differences between the controls and the test reactors were small. This suggests that any effects of PCP upon these anaerobic digesters (SRT = 73 days) were small. When the daily gas production rates were plotted as a function of time along with the averages of the control reactor means and standard deviations it was found that virtually all of the gas data from the PCP reactors fell within the range of the average control mean ± 2 standard deviations, which

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Table 16 Daily Gas Production in Liters At S.T.P.

LINEAR REGRESSION ANALYSIS ON TIME

		Mean	Standard Deviation	Slope	R^2
	R-10	0.977	0.114	-3.3×10^{-5}	0.0001
Control	R-11	0.964	0.109	-3.3×10^{-4}	0.0099
	R-12	0.966	0.114	$+1.6 \times 10^{-5}$	0.0000
	R-4	0.917	0.113	$+5.0 \times 10^{-4}$	0.0207
PCP	R-5	0.936	0.109	$+5.6 \times 10^{-4}$	0.0276
	R-6	0.945	0.114	$+8.2 \times 10^{-5}$	0.0005

is roughly the 95% confidence interval (see Figures 44-46). Thus it was concluded that the effect of PCP upon the gas production rate was of little significance. (An analysis of variance was not attempted because the reactors were still in a transition state and insufficient data were available to allow a time-series analysis). Furthermore, no local regions of lesser gas production were associated with higher concentrations of PCP. All of these data together with the data from aerobic testing which indicated that sorption was of minor significance, leads to the conclusion that acclimation to PCP had been achieved and thus the transition to Phase II testing was commenced.

Phase II Testing

Transition--

The SRT values chosen for Phase II testing were 10, 20, and 40 days. No sludge recycle was employed in Phase II so these values represent the HRT as well. Reduction of the SRT of an anaerobic digester increases the organic loading rate and stresses the system, which in itself could cause reactor failure. Thus the transition period from Phase I testing to Phase II testing was much more critical than it had been aerobic testing. As a consequence, several precautions were taken. First, since the PCP concentrations in the reactors had been cycling between the lower detectable limit and values of 400 to 500 μg/l, it was thought that initiation of the changeover when the PCP concentrations had just reached their minimum values would decrease the stress. Thus the transition was begun when the minimum PCP value was reached. Second, to help reduce the PCP loading rate to the shorter SRT reactors, the PCP concentration in the feed was reduced from 7.6 to 5.0 mg/l. Finally, the change in SRT was not made in one step, but was done gradually over a period of 21 days. The scheme for the transition is shown schematically in Figure 48.

Performance--

Upon completion of the transition period, reactor R4 (SRT - 10 days),

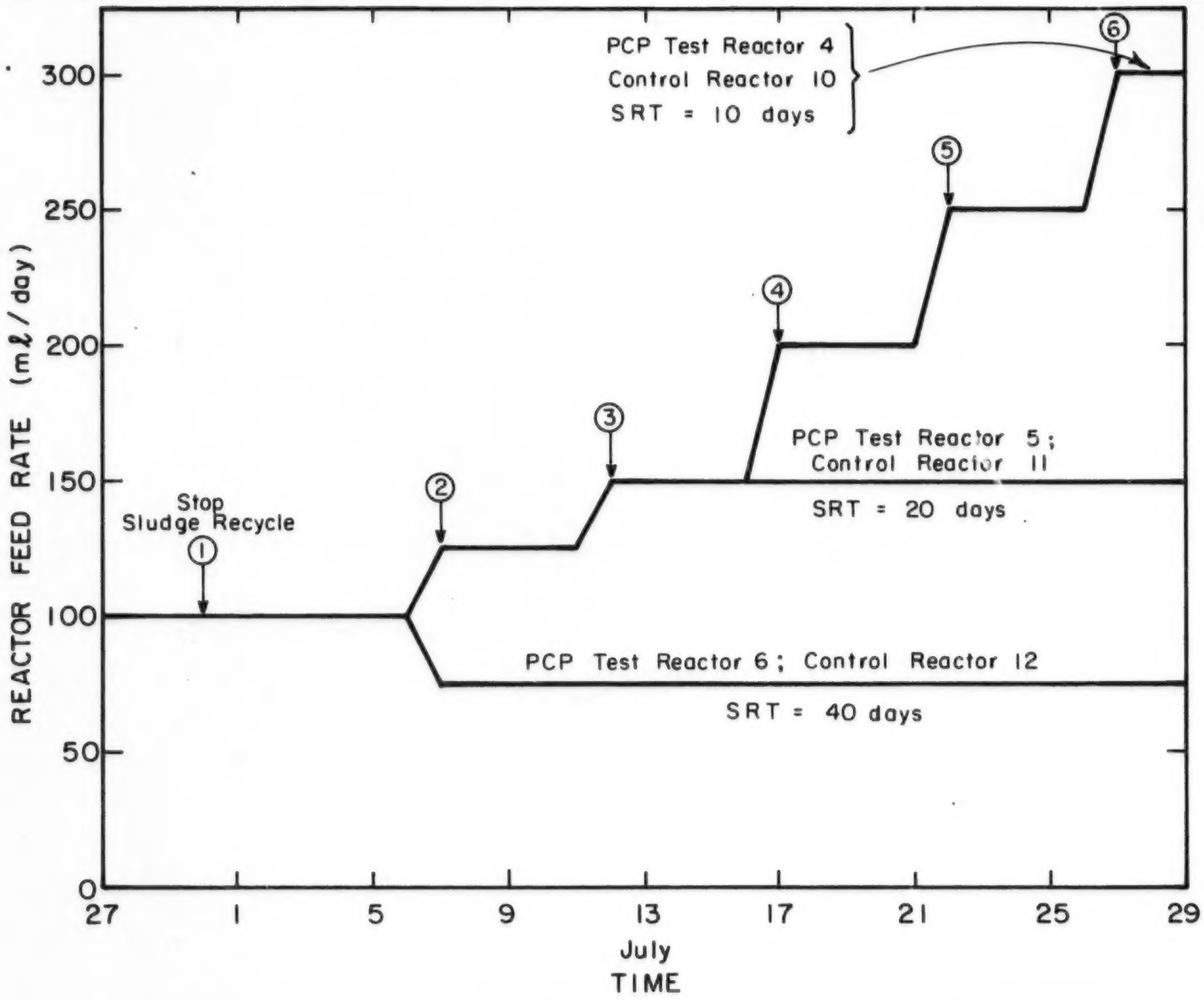

Figure 48 Scheme for the transition to Phase II. The numbers in circles correspond to the times at which the changes were made as shown in the next three figures.

R5 (SRT = 20 days) and R6 (SRT = 40 days) were fed once daily with raw sludge spiked with 5 mg/l of PCP as described in the methods section. Control reactors R10 (SRT = 10 days), R11 (SRT = 20 days) and R12 (SRT = 40 days) were supplied the same sludge with no PCP added. The operation of these reactors can be most easily observed by examination of Figures 49-51 which show a time oriented comparison of parameters examined for each control and PCP reactor. Furthermore, Figure 52 describes the gas production data for each of the three PCP reactors as ratios to the comparable control reactors. With respect to gas production and soluble PCP concentration, it appears that somewhat cyclic variations occured early in the test period but in all three reactors these early oscillations were dampened out and typical steady-state conditions persisted throughout the remaining experimental period. It should be noted that the time required for the oscillations to dampen out depended upon the SRT (and HRT) in the reactors with a longer time being required at the longer SRT's. It should also be noted that once the oscillations ceased the level of PCP in the reactors was below the detectable limit (~10 μg/l). Thus we have a situation in which PCP was reduced to very low concentrations with no periodic reduction in removal rate. Both of these occurrences are consistent with the theory that an intermediary degradation product had been the cause of the oscillations in the acclimation reactors, although additional work would be needed to prove that hypothesis. No confirmed explanation is available as to why it was not produced at these SRT values but a likely one is that the amount of inhibitory intermediate arising from an influent concentration of 5 mg/l PCP was not sufficient to cause the oscillations. An indication of the rather stable gas production characteristics of both the test and control reactors is summarized in Table 17 where it can be seen that the average daily gas production for the test reactors did not vary significantly from their untreated controls. As would be expected, gas production increased with decreasing SRT in both sets of reactors since the low SRT systems received higher daily increments of feed sludge. One must conclude from this study that PCP would not have any significant effect on gasification if digesters are operated within the SRT limits tested. It is probable however, that an anaerobic digester would be susceptible to PCP toxicity if shock loads of PCP were encountered, as evidenced by the experiences with the acclimation series clearly indicated that critical soluble levels of PCP in excess of 150 μg/l can have pronounced inhibitory effects on methane production which may progress to digester failure. Furthermore, once severe inhibition is established, the process of recovery is exceedingly slow. The toxicity test was valuable in the recognition of this problem and thus supports the decision to incorporate it into the protocol. Perhaps the protocol should be further modified to contain the provision that priority pollutants which show inhibition in the toxicity test should be first evaluated in Phase II at a non-toxic input level and subsequently be evaluated at a partially toxic level to obtain a clearer view of the impact of the pollutant on digester operation.

Further evidence that PCP had no effect upon digester performance under the conditions imposed in this study can be obtained by examining the other curves in Figures 49-51. In almost every case, once the transition had been accomplished and the oscillations in PCP concentration had disappeared, there was little or no discernable difference between the test reactor and the control. Thus one must also conclude that reactors operating at SRT values

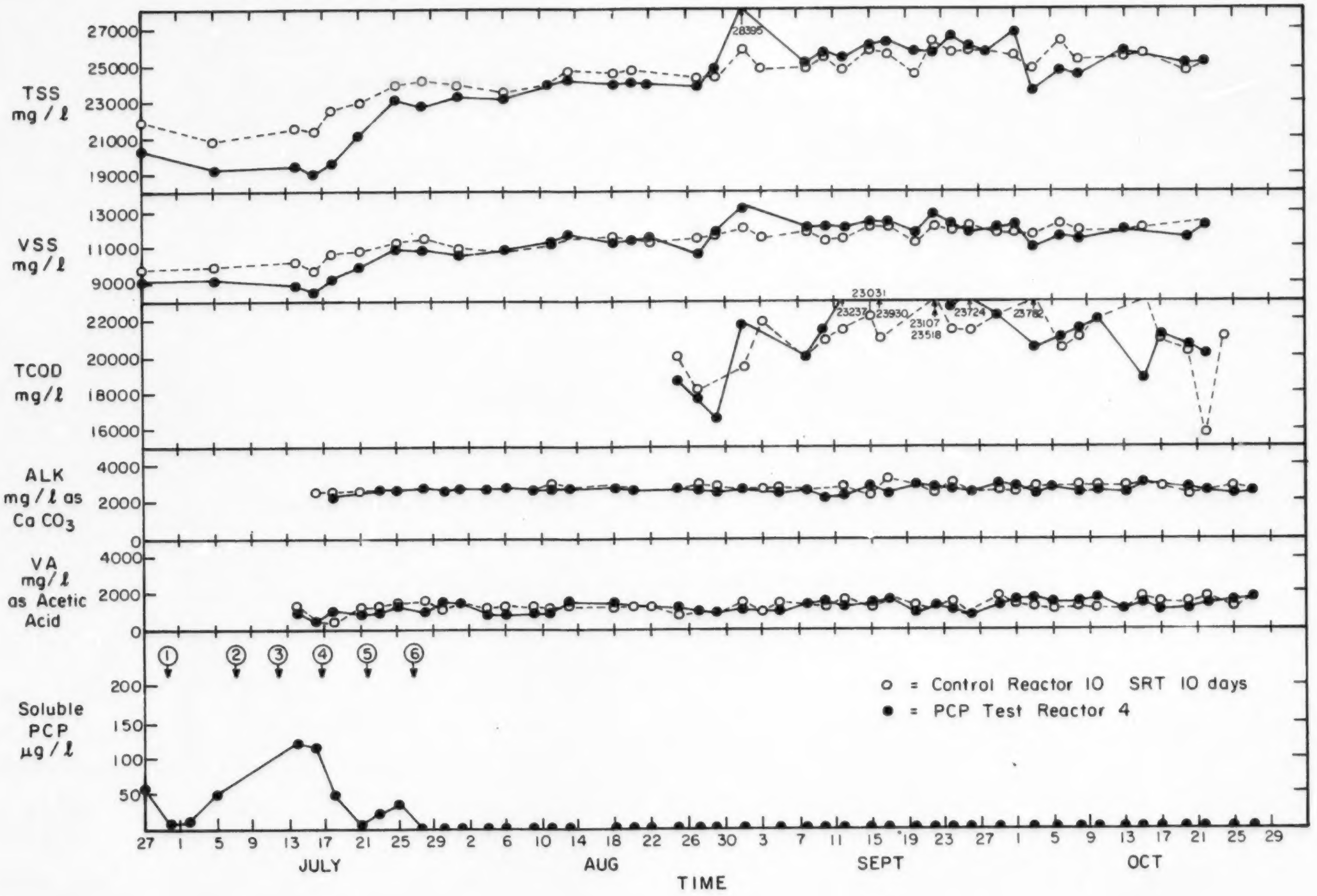

Figure 49 Comparative performance of reactors R4 (with PCP) and R10 (without PCP) when operated at an SRT of 10 days.

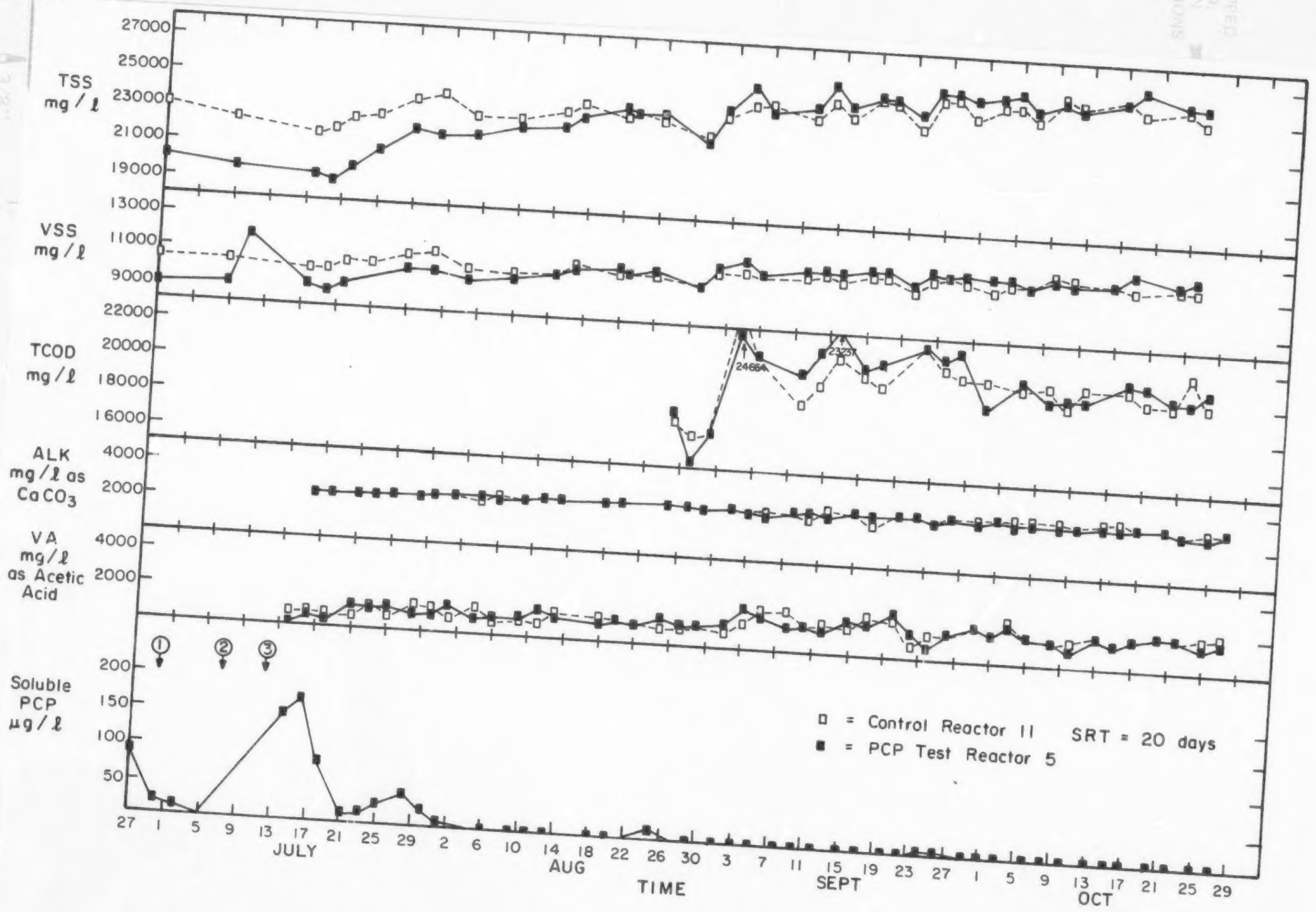

Figure 50 Comparative performance of reactors R5 (with PCP) and R11 (without PCP) when operated at an SRT of 20 days.

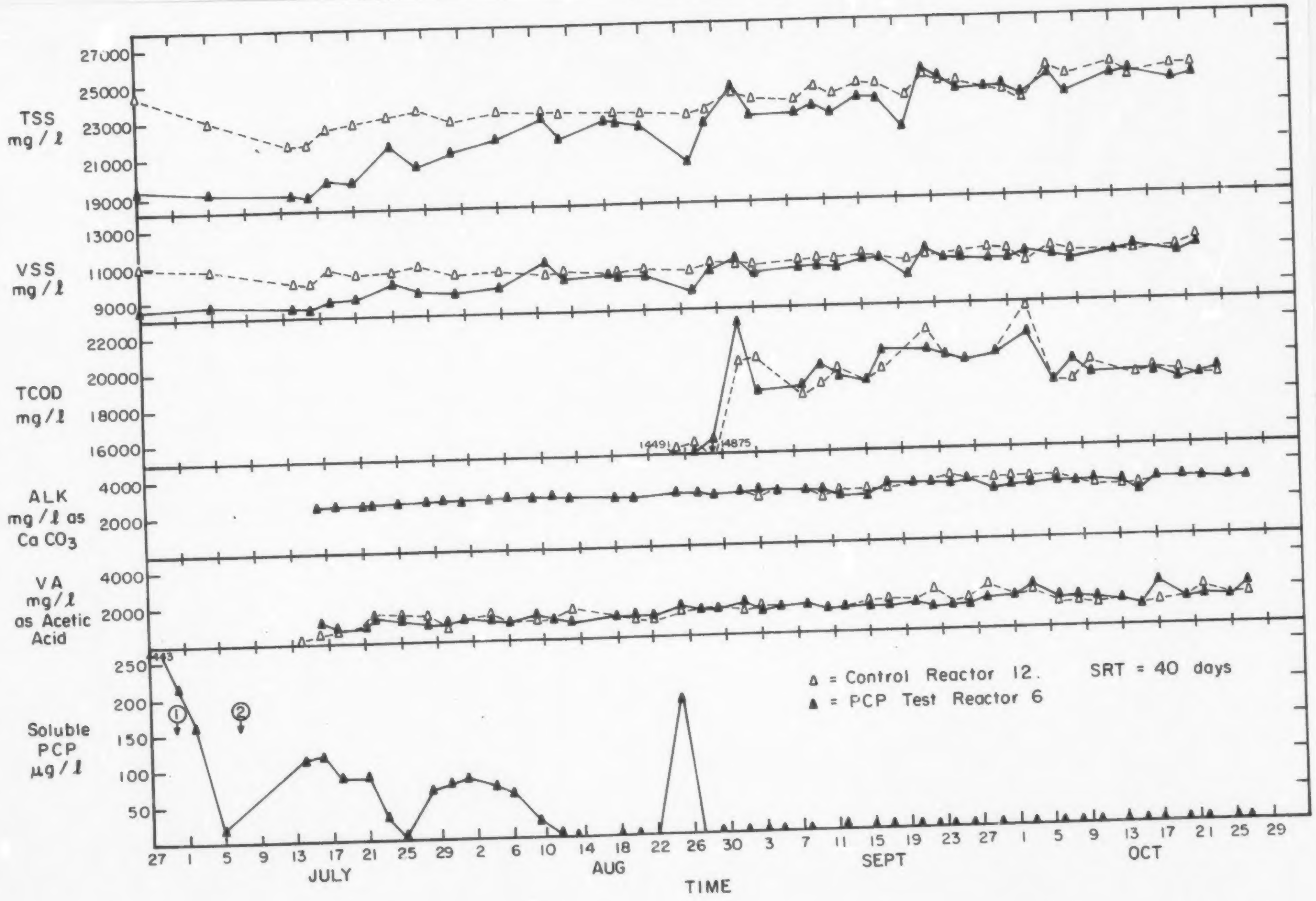

Figure 51 Comparative performance of reactors R6 (with PCP) and R12 (without PCP) when operated at an SRT of 40 days.

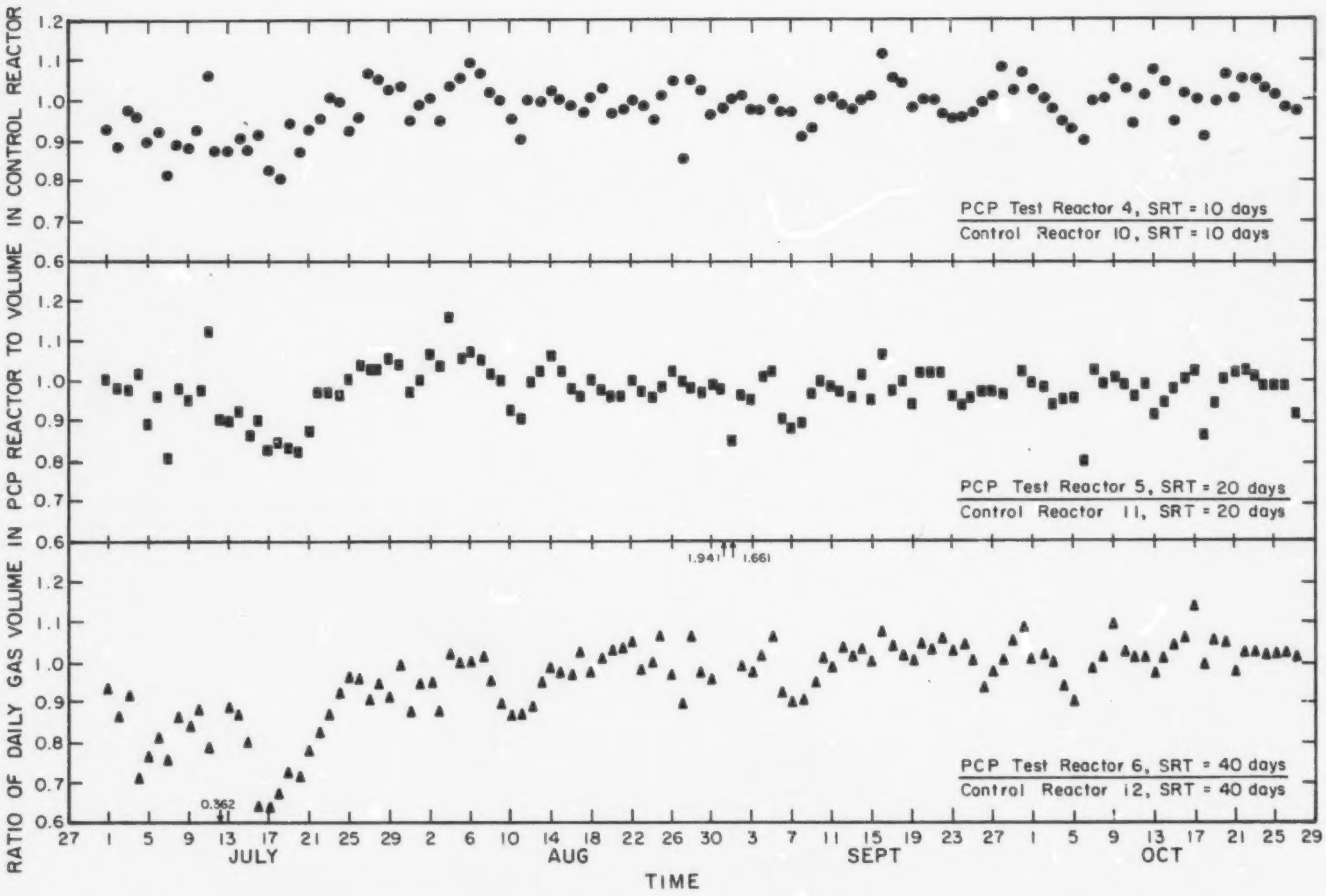

Figure 52 Gas production rate in reactors receiving PCP expressed as a fraction of the rate in the control reactors with the corresponding SRT.

from 10 to 40 days can tolerate 5 mg/l PCP in the influent sludge without any deleterious effect upon performance.

Table 17 Summary of Daily Gas Production for Control and Test Reactors

		Mean Gas Production	
	Reactor	1/day	std. dev.
R4	Test (SRT-10 days)	3.31	0.265
R10	Control (SRT-10 days)	3.32	0.227
R5	Test (SRT-20 days)	1.79	0.163
R11	Control (SRT-20 days)	1.84	0.140
R6	Test (SRT-40 days)	0.972	0.114
R12	Control (SRT-40 days)	0.956	0.129

In Chapter 4, where the rationale for this testing procedure was laid out, it was stated that an attempt would be made to correlate the concentration of the priority pollutant at steady-state with the SRT of the reactor. However, as discussed above, once steady-state was achieved the concentration of PCP was below the detectable limit of 10 μg/l. Although this prevents a correlation from being developed, it is an interesting finding for two reasons. First, it is interesting because the concentration in the anaerobic reactor at an SRT of 10 days is lower than the concentration in any of the aerobic reactors, even those with longer SRT values. Second, it is interesting because it suggests that PCP was undergoing very complete primary biodegradation. Before the extent of that biodegradation could be confirmed, however, alternative removal mechanisms had to be evaluated.

Evaluation of Alternative Removal Mechanisms--

Adsorption--The contribution of adsorption to the removal of PCP from the aerobic reactors was estimated by evaluating the isotherm for adsorption onto biomass and then assuming that the quantity of PCP adsorbed onto the biomass in the Phase II reactors was represented by the capacity in equilibrium with the steady-state PCP concentration in a reactor. Because of the continuous feeding of predominately soluble substrate to the aerobic reactors such an equilibrium value should give a reasonable approximation of the amount of sorbed material. Because of several factors, however, such a procedure was considered to be less reliable for the anaerobic experiments. First, primary sewage sludge contains a large amount of lipid material into which PCP is likely to partition, because of the lipophilic nature of PCP. Second, PCP was added to the raw sludge prior to feeding so that the sludge was contacted with 5 mg/l PCP (although for a very short time) prior to its dilution into the reactor. If irreversible sorption occurred, some of this material would not come back out of the sludge into solution and thus the solution concentration might not reflect the concentration on the solids like it would in a true equilibrium exchange. Third, the concentration of PCP in solution at steady-state was below the detectable limit which would imply that no PCP was sorbed to the sludge, a condition not likely to exist because of the first two factors. Consequently, it was decided that the best approach was to extract PCP directly from the sludge in the PCP reactors, thereby not relying upon an indirect measurement.

Having determined that direct extraction of the sludge was necessary, it then became necessary to calibrate the extraction procedure since the extraction efficiency was expected to be less than 100 percent. Furthermore, because of the possibility that the holding capacity of the acclimated sludge for PCP might be different from that of unacclimated sludge it was decided to use acclimated sludge in the calibration procedure. This required that the adsorption conditions be such that biodegradation would be inhibited, thereby allowing the amount of PCP applied and that remaining in the sludge-free liquor after a period of time sufficient for equilibrium to be achieved. Since it was likely that any chemical agent capable of inhibiting biological activity would also alter the sorptive properties of the sludge, it was decided that the best approach would be to use a low temperature. This decision was based upon the idea that a very low temperature would severely hinder the rate of biological activity while altering the sorption process much less drastically. To test this procedure for decreasing the importance of biological elimination a sorption experiment was performed at 4C and 37C with both acclimated and unacclimated sludge. The details follow.

Sludge was taken from both of the reactors which had a 10 day SRT and 100 ml of each was placed into two serum bottles under a N_2/CO_2 atmosphere. One bottle of each type of sludge was placed at each temperature. To test for sorption onto glass or possible reactions with the liquid, samples of liquid associated with the sludges were prepared by filtration through 0.45 μm membrane filters and treated similarly to give four additional controls. After temperature equilibration for three hours, 50 μg PCP (as Na PCP) was added to each bottle, giving an initial concentration of 500 μg/1. The bottles were vigorously mixed and then placed on shaker tables in the 4°C and 37°C rooms. Samples of 15 ml were taken, under N_2/CO_2 atmosphere, at 1.5, 5, 14, 23, and 46.5 hours. These were centrifuged at 4°C and soluble PCP was determined. Initial levels of soluble PCP were determined using filtrate samples.

The results of this experiment are shown in Table 18. It should be noted that there was a significant decrease in PCP concentration in both sludge samples at both the high and low temperatures within the first sampling period (1.5 hours). However, after 5 hours, the acclimated sludge reduced the concentration of PCP below detectable limits whereas fairly constant, small, but measurable concentrations of PCP were observed for the duration of the experiment at 4°C. The unacclimated sludge reduced the concentration to approximately the same extent as the acclimated sludge at 4°C. Note also that in the cell-free controls, the levels of PCP, although more variable than one might expect, were never reduced as substantially as in the sludge samples. It also should be noted that no attempt was made to keep these filtrates sterile after the biomass was removed and contaminating bacteria could possibly be present: therefore the data obtained were taken to mean that at 4°C biodegradation was substantially halted and that the loss of PCP observed was primarily the result of sorption. It also appears that holding

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Table 18 Effect of Temperature and Acclimation on Uptake of PCP

Sample	Temp. (C)	Soluble Concentration PCP (µg/1) Time in hours 1.5	5.0	14.0	23.0	46.5
Acclimated Sludge	37	19.6	0	0	0	0
	4	18.0	10.1	13.1	24.1	13.3
Unacclimated Sludge	37	40.7	20.9	27.3	15.3	11.2
Acclimated Filtrate	37	390	488	561	617	293
	4	341	364	459	490	355
Unacclimated Filtrate	37	407	532	394	363	373
	4	443	303	370	409	355

the acclimated biomass in contact with PCP for 5 hours is sufficient time to complete the sorption process.

Having established that the significance of biological activity could be reduced by performing the sorption test at 4°C, the next step was to use sludge which had been contacted with PCP at that temperature in order to evaluate the extraction efficiency. Consequently, triplicate samples of PCP-acclimated sludge were contacted with two concentrations of PCP, 5000 µg/1 and 500 µg/1, and the masses sorbed onto the sludge were calculated by difference. These samples were then extracted with the procedure outlined below.

1). After exposure of a known concentration of sludge to a known concentration of PCP at 4°C remove a sludge sample and place 40 ml in centrifuge tube.

2). Centrifuge at 12000 rpm (18400XG) for 30 minutes at 4°C.

3). Remove 10 ml centrate for PCP analysis.

4). Discard remaining centrate.

5). Place solid residue in 40 ml beaker, rinse tube with 5 ml deionized water and add to beaker.

6). Add 5-6 drops conc. H_2SO_4 to beaker mix with spatula to break up pellet.

7). Place contents of beaker into cellulose thimble filled about 2/3 full of the cellite filter-aid, rinse beaker twice with 5 ml portions of deionized water and add to thimble.

8). Place thimble in 108° oven for 6 hours.

9). Place thimble in soxhlet extractor and extract with hexane for 4 hours at 12 cycles/min.

10). Evaporate hexane extract from flask with a viqreux column on steam table or with air stream and occasional hexane rinsings of the flask walls. Concentrate to < 10 ml.

11). Add hexane extract to 125 separatory funnel and add hexane rinsings of flask to bring volume up to 10 ml (if volume is greater than 10 ml, evaporate hexane in funnel with an air stream).

12). Add 20 ml 0.125 N NaOH to separatory funnel and shake for 1 minute.

13). Allow layers to separate and add aqueous layer to another separatory funnel. Break emulsion mechanically until no more water can be recovered. Then add one spatula of KCl, shake, and drain the rest of the water into the separatory funnel.

14). Add 2 more scoops of KCl to the aqueous sample.

15). Add 20 ml 1% chloroacetic anhydride in benzene to water and shake vigorously for 2-3 seconds. Immediately drain aqueous layer and discard.

16). Add 20 ml 1.5% $NaCO_3$ to benzene fraction and shake vigorously for 10 sec.

17). Separate, drain and discard aqueous layer, add benzene layer to vial containing $NaSO_4$ to dry.

18). Analyze for derivatized PCP.

The results of the analysis are shown in Table 19. There it can be seen that the efficiency of extraction for the low concentration was approximately 20% whereas for the high concentration it was 35%.

The next step was to extract PCP from the sludge in the test reactors. The amount measured had to be divided by the extraction efficiency to estimate the amount in the sludge. Since the extraction efficiency varied with the amount of PCP on the sludge a decision had to be made concerning which efficiency to use. The concentration of PCP in the feed immediately prior to its addition to the reactor was 5000 µg/l; however, the contact time at that concentration was quite short. Immediately after feeding the concentration would be 500 µg/l, 250 µg/l and 125 µg/l, respectively. Thus the most realistic efficiency to use is 20%. The extraction procedure was applied to triplicate samples of the sludge from the reactors with SRT's of 10 and 20 days and to a duplicate sample of the sludge from the reactor with a 40 day SRT. The results in Table 20 show clearly that sorption was an insignificant mechanism.

Volatilization--When the mass transfer coefficient for the vigorously agitated anaerobic reactors was estimated it was found to be quite small. Since the anaerobic reactors were agitated with a magnetic mixer without

Table 20

Determination of PCP Removed by Sorption

Reactor No.	SRT days	Soluble Feed Rate 1/day	PCP Applied μg/day	Soluble PCP Conc. μg/1	Sorbed PCP μg/g**	TSS Conc. g/1	PCP Removed on Sludge	
							μg/day	% of applied
R1	10	0.300	1500	BDL*	1.3±1.5	25.5	10	0.67
R5	20	0.150	750	BDL	2.9±1.5	25.2	11	1.50
R6	40	0.75	375	BDL	0.78±0.58	24.5	1.4	0.37

*Below detectable limits.

**Mean t standard deviation of triplicate determinations except for R6 for which duplicate determinations were employed.

Table 19 Efficiency of PCP Extraction from Acclimated Sludge

Applied PCP Conc. (µg/l)	Applied PCP* (µg)	Soluble PCP (µg)	Sorbed PCP (µg)	Extracted PCP (µg)	% Sorbed PCP Extracted
5000	200	54.3	145.7	50.3	34.5
5000	200	71.4	128.6	45.9	35.7
500	20	3.2	16.0	3.6	21.4
500	20	3.5	16.5	2.8	16.9
500	20	4.6	15.4	3.4	22.1

*Mass PCP added to 40 ml sludge.

bubble aeration, it is certain that the mass transfer coefficient was much smaller. Furthermore, because the reactors were sealed, the gas space was likely to be in equilibrium with the liquid. Both of these facts suggest that the contribution to PCP by volatilization from the anaerobic reactors would be much smaller than from the aerobic reactors. Since volatilization made a negligible contribution to PCP removal from the aerobic reactors it is highly unlikely to have made a significant contribution in the anaerobic ones. Consequently, it was decided that attempt to measure its contribution would be a waste of time.

The nonbiological elimination mechanisms have been shown to have had a minor effect in the anaerobic reactors. Thus the elimination of PCP must have been by biological mechanisms. The determination of the extent of biodegradation was not part of the anerobic protocol and thus it was not attempted. Nevertheless, the fact that no inhibitory effects were named in the Phase II test reactors after steady-state suggests that the degree of degradation was probably in excess of primary.

Overall Evaluation of Protocol Effectiveness

The first attempt at Phase I testing (acclimation) demonstrated the necessity for tailoring the acclimation schedule to the particular priority pollutant under investigation rather than relying upon a standard schedule. This follows from the fact that methanogenic bacteria are quite sensitive to many organic compounds and from the key role that they play in anaerobic digestion. Furthermore, the type of reactor employed made consideration of this fact even more important. During aerobic testing a true CSTR was used with the result that the influent concentration of PCP had little effect upon the concentration in the reactor once degradation had begun. During anaerobic testing, however, semicontinuous reactors were employed with the result that the concentration immediately after feeding was equal to the concentration in the feed times the ratio of the feed volume to the reactor volume. For an inhibitory substrate the concentration in the feed must be selected so that the concentration in the reactor immediately after feeding does not exceed the inhibitory value.

When the anaerobic toxicity assay using PCP-unacclimated biomass was done, applied doses of PCP in excess of 200 μg/l resulted in decreased gas production rates. This suggested that the acclimation of biomass to PCP should be started at very low PCP input levels and the concentration increased very slowly. The guideline recommended is that PCP input should not exceed a concentration leading to equilibrium soluble PCP concentration in excess of 200 μg/l. Previous experience with PCP acclimation systems indicated that input concentrations of PCP in excess 6 mg/l resulted in soluble concentrations of PCP close to 200 μg/l. This was taken into consideration in the second acclimation attempt in which input PCP concentration was usually considerably less than 200 μg/l. When acclimation to 7.6 mg/l was achieved, it was noted that reactor PCP concentrations as high as 500 μg/l were tolerated without detrimental inhibitory effects. However, in order to avoid possible toxicity in second phase testing where reactor with lower SRT's were to be studied, a maximum input PCP concentration of 5 mg/l was employed.

Once Phase II testing was initiated the reactors ran quite smoothly and no troubles were encountered. In fact, the results indicated clearly that the reactors were able to tolerate the applied dose of PCP with no evidence of stress. Thus the protocol was successful in evaluating the effect of PCP upon anaerobic reactor performance. Although it was not the objective of this work to develop a kinetic relationship for disappearance of PCP in anaerobic digestion it was hoped that some correlation between SRT and PCP concentration could be obtained. This was not possible, however, because PCP underwent extensive enough degradation to reduce its concentration to less than detectable levels (10 μg/l). At this point in time, however, knowledge of the fact that PCP can be reduced to such low levels in anaerobic reactors with SRT's short as 10 days may be sufficient.

The protocol does not call for an attempt to determine the extent of anaerobic biodegradation. Thus it can only be stated that primary biodegradation occurred. During the course of the work, however, spurious peaks were observed on the chromatograms which disappeared as acclimation progressed. This could indicate that more complete degradation was occurring. Such degradation is likely the result of a consortium of bacteria and thus delineation of the extent of degradation is likely to be difficult.

No attempt was made to determine the influence of volatilization because of the results obtained during aerobic testing. Thus that portion of the protocol was not necessary.

The least satisfactory aspect of the protocol was the determination of the contribution of sorption. The efficiency of extraction was quite low and thus better extraction procedures will be needed, expecially for compounds that are more readily sorbed. Perhaps the techniques recommended by Warner et al. (16) will give better results, although they were considered during this work. Nevertheless, this aspect of the protocol will need additional refinement when compounds are encountered which give significant sorption. In spite of the low recovery efficiencies for extraction of the sorbed material, the contribution of sorption to PCP removal was consistent with that encountered in the aerobic system and this gives one greater confidence in the data.

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